Open-Water Movements of the Cutthroat Trout (*Salmo clarki*) in Yellowstone Lake After Displacement from Spawning Streams[1,2]

By Lawrence A. Jahn

Department of Zoology and Entomology
Montana State University, Bozeman, Montana

ABSTRACT

Cutthroat trout were taken from their spawning streams (June–August 1964 and 1965). A Styrofoam float was attached and fish were tracked from two release points. They moved generally shoreward (eastward). Of 120 fish tracked when the sun was visible, 68% went toward shore, 19% went away from shore, 4% showed random movement, and 8% were lost. Of 20 fish released when the sun was obscured, 30% went toward shore, 30% went away from shore, 30% showed random movement, and 10% were lost. Movement toward the eastern shoreline was less pronounced for fish liberated at a mid-lake release point. No directional preference was shown with respect to surface currents. There was no evidence that specific landmarks were used for orientation, but the sun may have served as a reference point since fish traveled farther and showed a stronger shoreward tendency on sunny days.

INTRODUCTION

The present study is concerned with "open-water" movements of the cutthroat trout (*Salmo clarki*) to see if fish displaced into Yellowstone Lake from tributary streams moved in definable patterns. Open water is defined here as that part of the lake where depths are sufficiently great to eliminate use of bottom cues for orientation. Research concerned with such movements has recently been undertaken on the white bass (*Roccus chrysops*) by Hasler et al. (1958) and Henderson (MS, 1963), but no previous studies are known on the open-water movements of cutthroat trout.

Various fishes show oriented movements with those of salmonids being perhaps the best known. Lindsey et al. (1959) found that adult rainbow trout (*Salmo gairdneri*) returned to their homestreams with very little straying, and Shapovalov and Taft (1954) reported similar results for the steelhead trout (*Salmo gairdneri gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Johnson and Groot (1963) discovered well-oriented movement of sockeye salmon (*Oncorhynchus nerka*) through a complex lake system, and Groot (1965) suggested sockeye smolts may have more than one direction-finding system used during migration. Stuart (1957) has shown repeat homing, i.e. year after year, and homing following displacement for brown trout (*Salmo trutta*). Platts (1959) found that artificially spawned cutthroat trout returned

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to their homestreams after displacement to a Utah reservoir. Ball (1955) showed that most adult cutthroat trout in Yellowstone Lake returned to their natal streams, and Cope (1956) reported diurnal variation in stream movements of this species.

Studies on vision and olfaction have shown them to be important for oriented movements of fishes. Investigations concerned with visual cues include Hasler et al. (1958), Schwassmann and Braemer (1961), Henderson (MS, 1963), Hasler and Wisby (1958), Schwassmann and Hasler (1964) and Groot (1965). Those concerned with olfactory cues include Hasler and Wisby (1951), Wisby and Hasler (1954), Hasler (1960), Idler et al. (1961), Brett and Groot (1963), and Fagerlund et al. (1963).

Yellowstone Lake has excellent conditions for movement studies because: it is large (362 km^2) and deep (97 m); it contains an endemic cutthroat trout population on which certain basic homing studies have already been made (Ball, 1955); it is not contaminated with trout from other sources, and has permanent fish traps in the mouths of Clear, Cub, and Pelican creeks (Fig. 1) thus making fish readily available for experimental purposes.

METHODS AND MATERIALS

Experiments were conducted during June, July, and August of 1964 and 1965. The cutthroat trout used were taken from the Clear Creek and Cub Creek traps. These were sexually mature and moving upstream to spawn. They were netted from the traps and immediately put into a covered tank containing

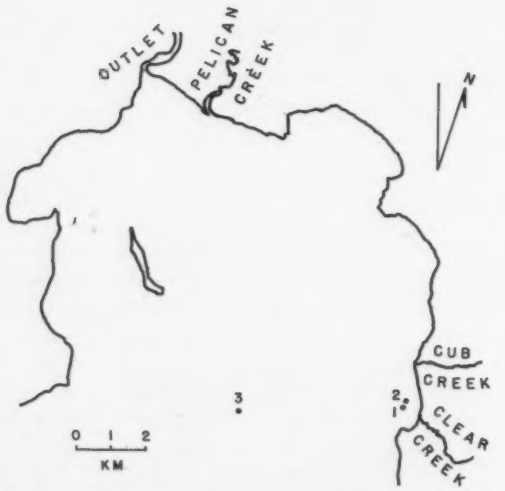

FIG. 1. The northern portion of Yellowstone Lake showing the locations of release points (○) and creeks where experimental fish were secured.

20–25 liters of creek water. This tank was transported to a boat and then to one of three release points in the lake (Fig. 1). It took 6–8 min to get to Release Points 1 and 2 and 14–18 min to Release Point 3. Release Point 1 was 0.56 km northwest (water 29 m deep) and Release Point 2 (water 29 m deep) was 0.50 km west-northwest of Clear Creek. Release Point 3 was 5 km directly west of Clear Creek where the water was 89 m deep. Release Point 1 was used in 1964 and Release Points 2 and 3 in 1965.

A tracking device was attached at the posterior margin of the dorsal fin of each experimental fish soon after reaching the release point. Fish were then carefully released as quickly as possible. Two trout were liberated at Release Points 1 and 2 and five trout at Release Point 3 for each experiment. Forty-two fish taken in Clear Creek were tracked from Point 1 (June 13 to July 30, 1964). Sixty-five fish from Clear Creek and 33 fish from Cub Creek were tracked from Point 2 (June 21 to July 19, 1965). Twenty-five fish from Clear Creek and 18 from Cub Creek were released at Point 3 (July 23 to August 5, 1965). Lengths and sexes of fish recovered after completed experiments in 1965 are given in Table I.

Three types of tracking devices were tried. The most successful was of the "bobber" type modified from that of Hasler and Wisby (1958), and only data obtained from this device were used. The float consisted of a Styrofoam (Dow Chemical Company) cube, about 5 cm on a side, covered with aluminum foil. This was fastened to one end of a 2-m nylon line which had an alligator clip at the other end for attachment to the fin of the fish.

The device was usually pulled underwater by the fish immediately after release. This condition persisted no longer than 90 sec, and then the float usually remained at the surface for the rest of the experiment. The float, having bright reflective surfaces, was clearly visible for about 0.5 km in calm

TABLE I. Lengths and sexes of experimental fish (1965).

Creek of origin	Release point	Number and sex	Length (*mm*)	
			Range	Average
Clear	2	16♂	340–400	366(10)[a]
		10♀		372(1)
Cub	2	21♂	320–390	362(21)
		9♀	320–360	347(9)
Clear	3	10♂	330–360	343(10)
		3♀	290–360	333(3)
Cub	3	10♂	340–390	366(9)
		6♀	350–370	362(6)

[a]Number of fish measured.

water. During 1964, the locations of experimental fish were determined by sighting on landmarks with the unaided eye. During 1965, a sextant was used which made observations more accurate. In order not to disturb the fish, it was necessary to remain at least 20 m away when recording locations. In final analyses, appropriate corrections for the distance between the boat and the fish were made in plotting the locations of the fish on maps. Plastic bobbers 6.35 cm in diameter, used by sports fishermen, were tried as floats on this tracking device, but these were less satisfactory than the Styrofoam floats.

Experiments originating at Release Points 1 and 2 were terminated after 2 hr. It was found early in the study that, if fish were not near shore by that time, their movements were either random or directed away from shore. Experiments were terminated sooner than 2 hr if fish reached water shallow enough so that a silver-colored disk (about 20 cm in diameter) could be seen on the lake bottom. When the disk could be seen it was assumed that fish might have been getting orientational cues from the bottom.

Positions of experimental fish liberated at Release Points 1 and 2 were plotted for 0.5, 1.0, 1.5, and 2.0 hr after release and at termination. Locations of some fish were not obtained at some of the designated times because they were temporarily "lost." From the plots, the direction of movement of fish from either release point was determined and the mean direction for each time interval calculated, according to the method described by Batschelet (1965).

Experiments originating at Release Point 3 were terminated after 1 hr, and the fish were picked up as quickly as possible. One sighting was taken on each fish, at the end of each experiment. This procedure gave information of straight-line distance and direction of "take-off."

A drift drogue suspended at 1 m by a heavy cord from a yellow float was used to determine currents at the approximate depth of the experimental fish. This device was made of two aluminum sheets 50 × 41 × 0.15 cm fitted together at right angles by means of a lengthwise slit halfway through each piece. Sightings on the drift drogue were made at the same intervals as those on experimental fish.

Another device tested for securing information on direction of take-off was that described by Horrall (MS, 1961). This consisted of a timing mechanism which triggered the inflation of a balloon and dragged the fish to the surface, where its position could then be determined after a desired period of time. This device had the advantage of not restricting the fish to the upper 2-m zone of the lake as did the "bobber"-type. An attempt to use this device in Yellowstone Lake was unsuccessful, probably because cold water and pressure affected its operation.

A third device for tracking consisted of a circular table marked in 10° intervals and a calibrated monofilament line attached to the experimental fish with an alligator clip. When the fish pulled out the line, this showed the distance traveled and also the number of degrees from north at any given time.

This device proved to be unsatisfactory because of its size and because wind caused bowing of the line.

RESULTS

The mean directions in degrees from true north (Fig. 2–4) of fish released from Points 1 and 2 showed them to have moved eastward. Clear and Cub Creek fish released at Point 3 (Fig. 5) moved northeast and north-northeast, respectively, after 1.0 hr.

During periods when the sun was visible, a strong tendency to move shoreward was noted (Table II) and often the fish swam in almost straight paths. Only a few fish were released during cloudy conditions but they showed a less pronounced tendency to go shoreward. These generally swam along straight paths also. The straight-line distances that fish traveled from their respective release points (Table III) were greater when the sun was visible than when it was obscured; averaging 122 m farther at 0.5 hr, 138 m at 1.0 hr, 177 m at 1.5 hr, and 258 m at 2.0 hr.

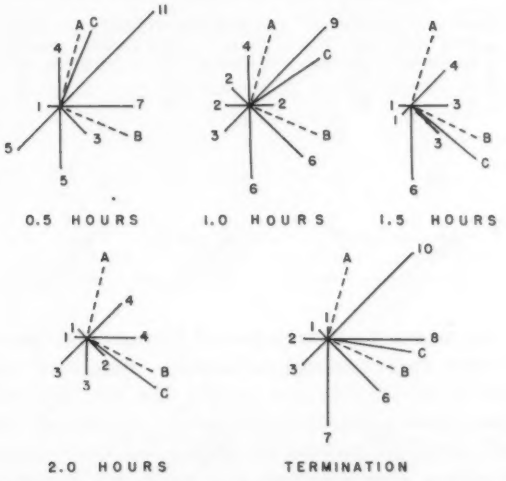

FIG. 2. Directions of movement of 42 Clear Creek fish from Release Point 1, 1964. Solid lines: directions of positions at the ½-hr intervals when records were taken, and when tracking of the various individuals ended; numerals: numbers of fish; A and B: directions of Cub and Clear creeks; C: mean direction for all fish. North — toward top of page. The numbers for which tracking was ended and of those lost at the various intervals were:

Hours	*Terminated*	*Temporarily "lost"*	*Lost*
0.5	3	6	0
1.0	9	4	1
1.5	8	8	3
2.0	18	0	0

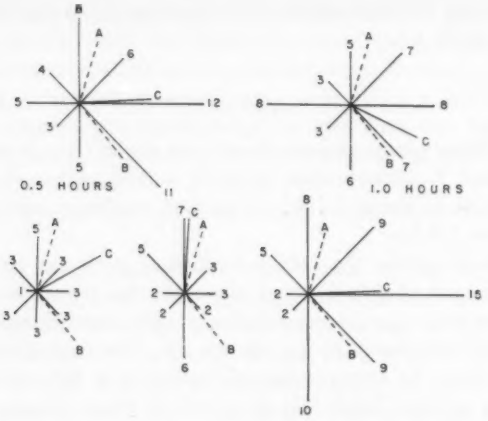

FIG. 3. Directions of movement of 64 Clear Creek fish from Release Point 2 (1965) (interpretation as in caption for Fig. 2). The numbers for which tracking was ended and of those lost at the various intervals were:

Hours	*Terminated*	*Temporarily "lost"*	*Lost*
0.5	10	10	0
1.0	15	6	1
1.5	5	11	3
2.0	30	0	0

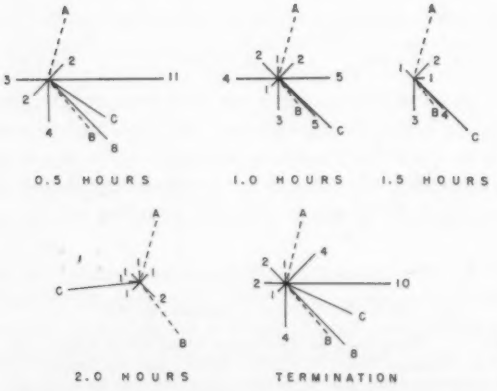

FIG. 4. Directions of movement of 33 Cub Creek fish from Release Point 2 (1965) (interpretation as in caption for Fig. 2). The numbers for which tracking was ended and of those lost at the various intervals were:

Hours	*Terminated*	*Temporarily "lost"*	*Lost*
0.5	8	4	0
1.0	8	2	1
1.5	9	6	0
2.0	7	1	0

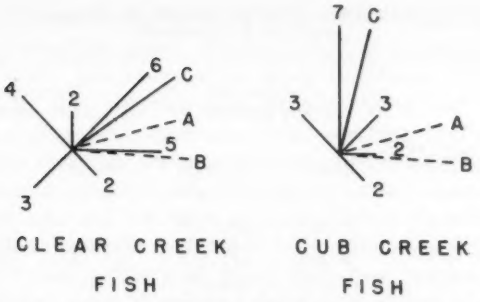

FIG. 5. Directions of "take-off" of 25 Clear Creek fish and 18 Cub Creek fish after 1.0 hr (interpretation as in caption for Fig. 2). Three Clear Creek fish and one Cub Creek fish were lost during the experiments.

TABLE II. Directions of movement of fish released from Points 1 and 2 when the sun was visible and when it was obscured.

Release Point	Creek of origin	Sun visible (+) obscured (–)	Number released	Number that moved			
				To shore	Away from shore	At random	Lost
1	Clear	+	39	27	5	3	4
		–	3	1	1	1	0
2	Clear	+	50	33	12	0	5
		–	15	4	5	5	1
2	Cub	+	31	22	6	2	1
		–	2	1	0	0	1

Experimental fish showed no significant difference in swimming speed toward or away from shore. Swimming speeds ranged from 584 to 731 m/hr in periods when the sun was visible and 397–555 m/hr when the sun was obscured. Fish showed no directional preference with respect to currents and usually swam much faster than the surface currents. The current drogue moved an average of 151 m/hr in a predominantly southerly direction from Points 1 and 2 while it drifted at an average speed of 209 m/hr northward from Point 3.

Seventeen fish tracked from Points 1 and 2 during the afternoons showed a southward shift in mean direction ranging from 45.6 to 140.4° farther southward than 111 fish tracked before noon (Table IV). Those tracked after noon still swam mainly toward shore as did the fish tracked before noon.

Females taken in Clear Creek traveled an average of 256 m farther from Release Point 2 than did males. The average time for females to reach shore was 57 min (range 26–122 min) while that for males was 59 min (range 33–141

TABLE III. Cumulative straight-line distances traveled (in meters) from Release Points 1, 2, and 3 at ½-hr intervals.

Release Point	Creek of origin	Hours after release	Distance in sunny weather		Distance in cloudy weather		Total average
			Range	Average	Range	Average	
1	Clear	0.5	15–1003	316 (35)[a]		173 (1)[a]	313
		1.0	130–1195	455 (32)	210– 495	352 (2)	448
		1.5	99–1003	545 (16)	142– 557	350 (2)	502
		2.0	68–1109	566 (17)		25 (1)	535
2	Clear	0.5	44– 804	337 (43)	87– 538	215 (11)	311
		1.0	99–1046	557 (34)	49– 792	367 (13)	505
		1.5	118–1442	619 (16)	185– 743	359 (8)	532
		2.0	160–1430	751 (21)	198– 725	389 (9)	643
2	Cub	0.5	117– 792	382 (29)		328 (1)	380
		1.0	106– 861	446 (21)	155– 409	282 (2)	432
		1.5	217– 984	574 (10)		861 (1)	601
		2.0	180– 954	414 (6)		594 (1)	439
3	Clear	1.0	226–1843	731 (14)	150–1655	555 (8)	667
3	Cub	1.0	75–1279	638 (17)			638

[a]Number of fish.

TABLE IV. Mean directions clockwise from due north traveled by fish tracked from Release Points 1 and 2 before and after noon.

Release Point	Creek of origin	Forenoon		Afternoon	
		No. tracked	Mean direction	No. tracked	Mean direction
1	Clear	32	97.7°	6	157.0°
2	Clear	53	88.1°	6	228.5°
2	Cub	26	113.1°	5	158.7°

min). Females taken in Cub Creek traveled an average of 111 m farther than males. The average time for these females to reach shore was 75 min (range 41–102 min) and that for males was 59 min (range 27–102 min). Females from both Clear Creek and Cub Creek released at Point 3 traveled an average of 81 m farther from the release point than did males.

DISCUSSION

The mean directions of movement for fish released at Points 1 and 2 were generally shoreward (easterly). The mouths of the creeks from which the fish were taken were also in this general direction. The experimental fish

were probably attracted to the streams to complete spawning or were simply attracted to the shoreline itself. Platts (1959) gives evidence for the first of these suppositions, but no previous work was found which supports the second. It may be that the shoreline provides the initial stimulus for the fish to swim in that general direction. After reaching the shallow water the fish might get cues directing them toward their spawning streams.

When released at Point 3, fish were about equally distant from east and west shorelines (5 km) and also from north and south shorelines (8 km). Clear Creek fish traveled northeast and had a mean direction of 38.4° north of the mouth of Clear Creek, while Cub Creek first traveled north-northeast and had a mean direction of 59.2° north of the mouth of Cub Creek. Statistically, the hypothesis that Clear Creek fish traveled in the direction of the homestream can not be rejected, but for Cub Creek fish it is rejected. However, only a small directional correction by Cub Creek fish would have been necessary for them to also head toward their homestream. It may be that fish taken late in the spawning season showed less "desire" to return to their streams to spawn. This may partially explain the lack of specific directionality toward their homestreams.

The use of specific landmarks for orientation immediately after release is doubtful. Everything seen in the atmosphere by the fish is compressed into the "fish window" (Walls, 1942), and therefore all objects become reduced in size. Only a small amount of light from these objects enters the water. Light coming from the highest landmark on the eastern shoreline as seen from Release Points 1 and 2 had an angle of incidence of 85°17′ and from the lowest prominent landmark (rise immediately above the beach) 88°18′. Thus, 60 and 84%, respectively, of incident light did not enter the water at Release Points 1 and 2. Similarly at Release Point 3, 71 and 89%, respectively, of the incident light failed to enter the water. It is doubtful if fish could recognize distinct objects on shore with the light that remained after dispersion and absorption occurred. However, the shoreline may appear as a thin band on one side of the fish window to which the fish might orient. Therefore, the possibility of distinguishing the shoreline was probably less when surface waves existed.

The tendency of cutthroat trout to go shoreward and the distances traveled from Release Points 1 and 2 were greater when the sun was visible than when it was obscured. Hasler et al. (1958) showed a tendency for white bass tracked in Lake Mendota to be randomly distributed under cloudy conditions. Winn et al. (1964) found that parrot fishes (*Scarus guacamaia* and *S. coelestinus*) circled, stopped, or lacked definite orientation when tracked under cloudy conditions. When the sun became visible, the parrot fishes began swimming in the appropriate direction of their offshore caves again. The sun seems to provide cues for the cutthroat trout, possibly as a reference point for orientation.

I have no explanation for the shift in mean direction of cutthroat trout tracked after noon. However, this might be explained by the change in the sun's position.

Future experiments using fish with blocked sensory pathways (such as vision and olfaction) should be useful in determining the mechanisms used in orientation. Night tracking should also indicate the importance of vision for oriented movement and perhaps the amount of interdependence of the senses for orientation.

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Response of Rainbow Trout (*Salmo gairdneri*) to Antigens of *Aeromonas hydrophila*[1,2]

By George Post

Colorado Cooperative Fishery Unit
Colorado State University
Fort Collins, Colorado

ABSTRACT

Rainbow trout (*Salmo gairdneri*) produced antibodies specific for *Aeromonas hydrophila*. Two parenteral routes yielded no measurable difference in rate of antibody synthesis. Method of bacterin suspension indicated adjuvant suspension was superior in maintenance of humoral antibody and challenge protection when compared to saline suspension of the organism.

Antibody synthesis from adjuvant-suspended bacterin lagged approximately 30 days behind the rate of synthesis by fish receiving saline suspended bacteria. The highest measurable antibody titer reached by an individual was 1:64. Individuals receiving saline suspended bacterin showed evidence of loss of titer approximately 2 months after the last antigen inoculation. Definite decrease in titer was noted 5 months after the last antigen inoculation and the return to a probable nonimmune class by approximately one-half of the individuals 7 months after the last inoculation. All individuals receiving adjuvant-suspended bacterin remained in the probable immune class for as long as 6 months following the last bacterin inoculation, but only approximately one-half of the individuals remained in the probable immune class 7 months postinoculation.

All fish receiving a weighed quantity of killed *A. hydrophila* orally each day for 9 consecutive months showed presence of humoral *A. hydrophila* antibody. This immunization procedure produced 50% of the individuals with humoral antibody titers considered to be in the probable immune class.

Fish receiving no immunization remained with negative antibody titers throughout the experimental period.

Definite protection was given fish which had been immunized by parenteral inoculation.

Similar protection was given after immunization by either intramuscular or intraperitoneal routes. Fish receiving either saline or adjuvant-suspended bacterin parenterally showed analogous protection when challenged by the specific bacteria. Fish receiving oral bacterin received questionable protection against parenteral challenge of the specific organism.

Phagocytosis was found to occur to a greater extent in immunized rainbow trout than in nonimmunized rainbow trout. An average phagocytic index of parenterally immunized rainbow trout was 1.08. Nonimmunized rainbow trout of the same age and egg source had an average phagocytic index of 0.30. The opsonic index was 3.6.

INTRODUCTION

The immune response of many animals to bacterial antigens has received much attention since Koch proved certain diseases are caused by specific microorganisms. The greatest portion of research and accomplishments in the field of immunology has been performed on homothermic animals. Only

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cursory examination of the immune responses stimulated by bacterial antigens in poikilothermic animals has been made.

Basic research in immunology performed on homothermic animals, and especially on man, has found application through techniques used to produce resistance against disease without danger of pathological infection with the disease. Immunizing techniques applicable to poikilothermic animals have not been devised.

Two species of bacteria (*Aeromonas hydrophila* and *A. liquifaciens*) are usually associated with "Red mouth" disease of certain fishes. The disease is present in many areas of the United States where trout, salmon, and chars are reared. The causative bacteria also produce a similar systemic disease in amphibians and reptiles. An outbreak of the disease in a population of fish can be controlled by therapeutic drugs. When the drug is withdrawn, the mortality rate among these fish may begin to rise within a short time. This recurrence of disease in fish which had previously been in contact with the causative organisms indicated little or no immune response among the fish.

Research was conducted, using *A. hydrophila* as an antigen, to demonstrate specific antibody production in rainbow trout. Both oral and parenteral delivery routes were used. Rate of antibody buildup was established. Protective immunity was determined by challenge with the viable pathogen. Rate of antibody titer reduction was determined through postinoculation surveillance.

MATERIALS AND METHODS

Facilities for performing experimental work consisted of cement-lined tanks. The water temperature was uniformly 15.5 C.

Rainbow trout (*Salmo gairdneri*) of the Hull strain were used as test animals. These fish were 19 months old at the beginning of the experiment. One-hundred fish (9–11 inches in length) were counted into each of five tanks at the initiation of the experimental procedures. There were approximately equal numbers of male and female fish.

Aeromonas hydrophila strain number 9071, American Type Culture Collection, was procured as the test organism. Bergey's Manual of Determinative Bacteriology (Breed et al., 1957) was the authority used to establish continuous identification of the organism throughout the experimental period. Subcultures of the organism were maintained on trypticase-yeast extract agar.

Antigens for parenteral injection were prepared just prior to being used. Cells of *A. hydrophila* were grown on trypticase-yeast extract agar, washed free of media with isotonic saline, and heat-killed. The bacterial suspension was adjusted with saline or Freund's complete adjuvant so that each 1 ml contained the equivalent of 1 mg of dry weight bacteria. Determination for sterility was made on all antigens before being used.

Oral bacterin was prepared by growing and harvesting *A. hydrophila*. The organisms were washed with saline and heat-killed. One-tenth per cent agar was dissolved in the suspension while the organisms were being heat-killed. The desired amount of dry, pelleted fish food was spread on a table and the suspended bacteria sprayed evenly on it with an atomizer and air pressure. The purpose of the agar was to cause the bacteria to adhere to the food pellets. The treated food pellets were dried at room temperature.

Antigens for agglutination tests were prepared from saline-washed and heat-killed *A. hydrophila*. The final saline suspension was adjusted so that a 1:8 dilution yielded an optical density of 0.35 using a Beckman DU spectrophotometer with an air blank, 0.1-mm slit, 520 mμ wavelength and 1.0-cm light path. Phenol was added to 0.5% concentration. The antigen was diluted 1:8 with saline as needed for agglutination tests. The stock antigen was stored at 4 C. New antigen was prepared at 4-month intervals.

Saline-suspended bacterin was injected into each experimental fish at approximately each 30-day period. Seven monthly injections of 1 ml of bacterin were given in all. Adjuvant-suspended bacterin was given three times during the inoculation period. Each inoculation of 1 ml of bacterin was given 2–2½ months following the previous inoculation. Oral bacterin was given with the diet on the basis of 1 mg of dry-weight bacteria for each fish each day. A total of 272 (9 months) daily feedings was given.

Intramuscular inoculations of either saline- or adjuvant-suspended bacterin were given into the muscle of the back at the base of the dorsal fin. Intraperitoneal inoculations were given through the pelvic girdle into the body cavity.

Blood was taken for determination of antibody level by cardiac puncture. Tricaine methane sulfonate (M. S. 222) was used to immobilize the fish before bleeding. Approximately 2 ml of blood were withdrawn at each bleeding. Bleeding intervals were approximately 30 days.

Blood samples were allowed to clot and the clots to retract at room temperature. Agglutination tests with the sera were set up using a twofold dilution of each serum starting at 1:2 dilution. A saline control and a negative serum control were included in each series of agglutination tests.

The agglutination tests were held at room temperature for 2 hr and at 4 C for an additional 22 hr. Each tube was then evaluated for agglutination.

The relative virulence of *A. hydrophila* to the experimental animals was determined by inoculation of serial dilutions of the living bacteria into nonimmunized fish. Ten passages of the organism through rainbow trout indicated that an LD_{50} would be a large number of bacteria. Therefore, bacterial dry weight was used as a basis to establish the LD_{50} (approximately 4×10^9 organisms/mg). One milliliter volumes containing the desired weight of bacteria were inoculated intraperitoneally into groups of four nonimmunized fish.

Mortalities were recorded for 2 weeks. Graphic estimations were made of lethal-dose levels of the organisms (0.39 mg dry weight bacteria equalled LD_{50}).

Fish from a specifically immunized group were chosen to establish immune and nonimmune class titers. An LD_{50} of viable *A. hydrophila* was injected intraperitoneally into six groups of eight fish with antibody titers of ± at 1:2 dilution up to an antibody titer of 1:32. No mortality was observed in fish with antibody titers of 1:4 or higher. Data from the immunization experiment were subjected to statistical evaluation by grouping titers of −, ± and 1:2 into a probable nonimmune class and titers of 1:4 or higher into a probable immune class.

Challenge experiments were conducted in cement-lined tanks. Ten fish of approximately equal size from each group of immunized fish and 10 fish of approximately equal size from the control (nonimmunized) group were placed in separate tanks. A quantity of saline suspended *A. hydrophila* equal to an LD_{90} (0.71 mg of dry weight bacteria) was inoculated intraperitoneally into each fish. Mortalities were recorded for a 14-day observation period. Necropsy and bacterial culture were performed to establish presence of *A. hydrophila*.

Demonstration of phagocytosis was more difficult than at first expected. *In vitro* phagocytosis could not be demonstrated. A procedure using intraperitoneal injections of glycogen served to demonstrate the phagocytic phenomenon.

Immunized and nonimmunized fish were given 1 ml of 0.01 mg/ml glycogen solution intraperitoneally. Twenty-four hours later a saline suspension of *A. hydrophila* was given intraperitoneally. Three and one-half hours later each fish was killed and impression smears prepared from the peritoneal wall. These smears were stained with Giemsa stain. Phagocytosis was observed on these slides. One-hundred phagocytes (heterophiles and macrophages) were counted from each fish. The average number of bacterial particles were calculated per phagocyte (phagocytic index). Phagocytic indices were prepared from immunized and nonimmunized fish.

RESULTS

The data on antibody titer were used to calculate slopes for both the probable immune and probable nonimmune classes for each route of bacterin delivery. Low slope values indicate relatively slow antibody synthesis in that group of fish. Higher slope values indicate more rapid antibody buildup (Table II).

Results of challenging immunized rainbow trout with living *A. hydrophila* are given in Table III. Each challenged group included 10 fish. These data represent the challenge information on 200 individual fish.

The average phagocytic index of 15 immunized fish was 1.08 (range 0.7–1.55). The average phagocytic index of 15 nonimmunized fish was 0.30 (range 0.11–0.48). Analysis of variance comparing the phagocytic indices of the two groups showed $t = 10.954$ which is a highly significant variation at the 0.01% level. Calculations using the average phagocytic indices of the two groups yielded a 3.6 opsonic index.

DISCUSSION

A review of literature pertaining to the immune response in fish and other poikilothermic animals brings out two main points: (1) the immunology of these animals has received little study; (2) the immune response in these animals is closely related to the environmental temperature (Nybelin, 1935; Pliska, 1939; Smith, 1940; Gee and Smith, 1941; Klontz, MS, 1959; Krantz et al., 1964). These references show that poikilothermic animals are definitely capable of producing antibodies against bacterial antigens. However, the environmental temperature plays an important role in this response; the body temperature of these animals is the same in quiescent animals, or only slightly increased in active animals, as is the environmental temperature (Clausen, 1934).

Most invasive pathogens, especially the bacteria, will cause bacteremia in poikilothermic animals. These animals are apparently unable to wall off infections and hold causative organisms to one or a few body organs, as in higher animals. Possibly there is some physiological reason, or possibly the reason lies entirely in the relationship of body temperature. The invasiveness of pathogenic bacteria of these animals is closely correlated with temperature. Perhaps there is little need for physiological or histological mechanisms which would remove invading bacterial organisms at low body temperatures. The generation time of the bacteria would be extended at low temperature to the point where propagation and destruction (or bacterial death from all cause) would be equal. Bacteria at this stage may maintain a constant population level within the tissue without invasion of other body tissue. The immune response of the animal would remain quiescent while the bacteria were present in the tissues of the animal at the lower temperature (near dormancy of the bacterium). An increase in environmental temperature would decrease the bacterial generation time. The balance of physiological destruction of bacteria and bacterial increase would be altered to the advantage of the parasite. Defense mechanisms of the animal would be overwhelmed. The first manifestation would be a bacteremia. The main defense of the animal against the pathogen would be more the inherent resistance than the acquired resistance.

The rainbow trout strains which are presently being cultured throughout much of the United States, as well as other areas of the world, are susceptible to invasion and bacteremia by the bacterial organism *A. hydrophila*. The American Type Culture Collection *Aeromonas hydrophila*, strain number 9071, seemed the logical choice of organism for the research reported here. This strain of organism is easily acquired by an investigator. However, this strain was found to be quite avirulent to the Hull strain of rainbow trout.

Results obtained, using all prepared bacterins on specific groups of fish, indicated production of humoral antibodies which could be demonstrated in the serum by agglutination tests performed against the specific antigen. Antibody titers in parenterally inoculated fish began rising to probable immune titers in most fish by the middle of the third month following immu-

nization. Titers increased slowly in most fish throughout the 6-month immunization period and were maintained for approximately 2 months following the last inoculation. Titers began to recede in most fish which received saline suspended bacterin by 4 months postinoculation. The fish receiving adjuvant-suspended bacterin maintained antibody titers up to 4 months following the last inoculation. All parenterally inoculated fish had lost much of their antibody titer by approximately 7 months postinoculation. Nearly 50% of these fish had become deficient enough in antibody titer at that time to be classified as probable nonimmune (Table I). Challenge experiments brought this out quite well (Table III).

Individuals in the orally inoculated group developed antibody titers against *A. hydrophila* in the blood, although a classification of probable immunity was reached by only 50% of the fish. This finding does show that an oral method of immunizing rainbow trout is possible.

Slope value indicated the intraperitoneal route of delivery of the bacterin was the most rapid method of producing protective antibody titers in these fish. Saline-suspended bacterin which was delivered intramuscularly had a slightly higher slope value than did the adjuvant-suspended bacterin delivered by the same route. Apparently bacterial antigens were absorbed more rapidly from saline suspension. The slope values also showed the slow response of the orally inoculated group of fish.

Parenteral challenge was found to be an acceptable method for determination of protective immunity in fish receiving antigen stimulation parenterally. Fish in these groups at the height of their humoral antibody titers were protected quite well from an LD_{90} of the specific bacteria. Usually 10–30% mortality was received on the high-level challenge. On the other hand, humoral antibody was apparently not an indication of actual immunity in the orally immunized fish. Sixty to 80% mortality was observed in fish which showed 40–50% of the individuals in the probable immune class (Tables I and III), while fish from the control (nonimmunized) group had 80–90% mortality.

Increased phagocytosis of bacteria which are specific to a previously given antigen has long been considered a method of measuring the immune response of an animal. Such should be the case in fish as well. Methods of determination of phagocytic index came under observation. No method of determining *in vitro* phagocytosis could be found. Time, temperature, cell agitation, varying anticoagulants, or concentration of phagocytes had no effect on phagocytosis. Phagocytosis could not be demonstrated when living specific bacteria were inoculated into the blood stream of immunized and nonimmunized fish. Mobilization of the phagocytic cells into the peritoneal cavity followed in 24 hr by inoculation of saline-suspended specific bacteria produced phagocytosis within $3\frac{1}{2}$ hr. The standard procedure for homothermic animals is similar but allows $\frac{1}{2}$ hr for phagocytosis. Many biological functions of animals are probably much reduced at 15.5 C; phagocytosis is probably one of those retarded functions, thus the need for $3\frac{1}{2}$ hr rather than the $\frac{1}{2}$ hr used for higher animals as a standard procedure.

TABLE I. Per cent of fish in the probable immune class throughout the study period.

Route and antigen preparation	Time in months from initial inoculation									
	0	2	3	4	5	6	7	8	11	14
Intramuscular saline suspension	0[a]	10[a]	80[a]	90[a]	100[a]	100[a]	100	100	80	55
Intramuscular adjuvant suspension	0[a]	0	85[a]	100[a]	100	100	100	100	100	55
Intraperitoneal saline suspension	0[a]	30[a]	75[a]	90[a]	100[a]	100[a]	100	100	80	45
Oral food suspension	0[b]	0[b]	15[b]	20[b]	25[b]	35[b]	50[b]	50[b]	35	25
Control	0	0	0	0	0	0	0	0	0	0

[a]Each fish received 1 ml of appropriate antigen on or about date given.
[b]Each fish received 1 mg of dry weight specific bacteria each day for 262 days.

TABLE II. Slope values for fish in the probable-immune class.

Route	Suspension	Slope
Intramuscular	Adjuvant	3.050
Intramuscular	Saline	3.133
Intraperitoneal	Saline	3.717
Oral	Food	1.300

TABLE III. Per cent mortality of fish on challenge with viable *Aeromonas hydrophila*.

Route for immunization	Method of suspension	Months following initial bacterin inoculation			
		6	7	8	14
Intramuscular	Adjuvant	10	20	20	70
Intramuscular	Saline	30	20	20	90
Intraperitoneal	Saline	20	20	20	90
Oral	Food	70	70	60	90
None[a]	–	80	90	80	100

[a]Control group (nonimmunized); expected mortality: 90%.

The opsonic index of 15 immunized and 15 nonimmunized fish showed an average of 3.6 times more phagocytosis occurring in the immunized fish. Analysis of variance conducted on the data collected on the two groups of fish showed the phagocytic indices of the immunized fish to be significantly higher at the 99% level of risk with 29 degrees of freedom.

The data reported here are an indication that fish can be immunized against specific diseases. Techniques of establishing antibodies within the fish must be found. Artificial immunization has been used for other domesticated animals to control disease. Similar procedures will be useful in the culture of fishes as well.

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Experimental Determination of Food Consumption of pike, *Esox lucius*, for Growth and Maintenance[1,2,3]

By L. Johnson[4]

Fisheries Research Board of Canada
Arctic Biological Station, Ste. Anne de Bellevue, Que.

ABSTRACT

Under laboratory conditions simulating those in the field, there were seasonal changes in both growth and in maintenance requirements of pike (*Esox lucius*), which were to some extent independent of temperature. The peak maintenance requirement of about 45 mg of minnow (*Phoxinus phoxinus*) per gram of pike occurred in late June, followed by a decline through August and September until a fairly steady state of about 25 mg/g was reached in October. Maintenance continued at this level until April when there was a gradual rise to the June peak. The maintenance coefficient was 1.385 g per gram per annum, and there was no apparent decrease with size. Growth also reached a peak in June then declined through the rest of the summer and fall until it ceased in midwinter. Growth resumed in March, the rate gradually increasing until the June peak was reached. After maintenance requirements have been met, 1 g food produces .437 g pike when feeding is *ad libitum*. The maximum value of the coefficient of utilization is .485, at a level of feeding below that of maximum intake. The efficiency of utilization at the maintenance level is .476. Comparing the results with those reported for other species shows that pike have relatively low maintenance requirements and relatively high values for the conversion of food to pike substance.

INTRODUCTION

Windermere is the largest lake in England and is situated in the northwest part of the country in the Lake District. It is the site of the Freshwater Biological Station, consequently the fish populations have been subjected to considerable scrutiny. With this background it was possible to plan a program to determine the food consumption of the pike population, first by investigating consumption under controlled conditions, and then by applying these results to the resident population. This paper describes the experimental procedure and results and another one (Johnson, 1966) describes the application of these results.

The food of pike in Windermere has been described in detail by Frost (1954); perch (*Perca fluviatilis*) is the mainstay of the diet although trout (*Salmo trutta*) and char (*Salvelinus alpinus willughbii*) are taken in some quantity during the winter months. Sticklebacks (*Gasterosteus aculeatus*) and minnows (*Phoxinus phoxinus*) form a subsidiary food source, particularly in the summer.

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[2]This work was carried out in part fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Leeds.
[3]This in the first of two parts of a study on food consumption of Lake Windermere pike; the other paper appears in this issue of the Journal.
[4]Present address: Fisheries Research Board of Canada, Freshwater Institute, Winnipeg, Man.

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The general picture that emerges is that pike tend to prey on whatever species is available in the relatively shallow waters which they inhabit.

Pike are generally thought to be rapacious predators capable of great digestive prowess. One authority (Cholmondley-Pennel, 1865) considered that one animal could consume twice its own weight of food in the space of a week. This view seems quite prevalent amongst anglers, probably in part due to the forcefulness with which pike take their prey.

In feeding experiments it is customary to separate the food required for maintenance from that required for production or growth. The maintenance requirement is that amount which must be consumed to keep the animal at constant weight. The energy so supplied is used for tissue maintenance, muscular activities associated with respiration, and for holding position against the force of gravity. The food for growth is assumed to be that amount which is consumed in excess of the maintenance requirement, although, particularly in fish, this is complicated by the production of eggs and milt which are subsequently voided from the body. In experimental work this factor may be overcome by the use of immature animals.

The maintenance requirement has been defined by Beverton and Holt (1957) as a function of the body weight:

$$\frac{d\xi_m}{dt} = \zeta w^j$$

where ξ_m is the annual consumption of food for maintenance, w is the weight, ζ is the annual maintenance coefficient or the maintenance per unit body weight, and j is an exponential coefficient equal to or less than 1; if less than 1 it implies that the maintenance will diminish as the size of the animal increases.

Growth is more difficult to define in mathematical terms since, for fishes, an expression is required which will take into account annual cyclical changes in growth where each succeeding cycle is less than that of the preceding one (Brown, 1957). Two different aspects of the problem may be considered: (1) short-term changes, that is, periods of 1 year or less, and (2) changes which take place over a period of years. Brown considers that short-term changes may be best expressed by the specific growth rate per cent per unit time (G):

$$G = \frac{(\mathrm{Log_e}\, Y_T - \mathrm{Log_e}\, Y_t)100}{T - t}$$

where Y_T and Y_t are the sizes (length or weight) at times T and t.

Beverton and Holt are of the opinion that the von Bertalanffy equation (von Bertalanffy, 1934, 1938), whilst not ideal, comes close to satisfying the essential requirements for a growth equation. From the initial equations can be derived expressions which give the length (l) and the weight (w) at time t:

$$l_t = L_\infty - (L_\infty - L_0)\, e^{-Kt}$$

$$w_t = \left\{ W_\infty^{1/3} - (W_\infty^{1/3} - W_0^{1/3})\, e^{-Kt} \right\}^3$$

where W_∞ and W_0 are the weights corresponding to lengths L_∞ and L_0 (W_∞ and L_∞ are weight and length at the growth asymptote) and K is a constant related to the rate of katabolism (von Bertalanffy constant).

Whilst it is highly probable that maximum growth will be obtained on *ad libitum* feeding, Brody and Proctor (1943) and Kostitzin (1939) consider that more efficient utilization of food takes place at some level of intake below the maximum. Thus, to obtain a reasonably complete picture of nutritional requirement in an animal it is necessary to examine the food consumption for maintenance and maximum growth as well as for levels of feeding between these two extremes.

METHODS

The methods have been designed to obtain the necessary information to provide a numerical solution for the equations derived by Beverton and Holt for the food consumption, annual production, and yield of a fish population.

The experimental pike were kept outside in large galvanized iron tanks measuring 1.25 × .75 × .75 m. The insides of the tanks were coated with bituminous paint and the water was regulated to a depth of 50 cm. Ten tanks were used with a water supply arranged in series, the first tank in the series holding the stock fish. A very small supply of water was introduced into the stock tank which then supplied water to the other tanks at the ambient temperature. The tanks were kept under beech trees which provided a dense shade in summer; apart from this shading from direct sunlight no attempt was made to control temperatures.

Cover for the pike was provided by the addition of liberal quantities of the aquatic moss *Fontenalis antipyretica* which maintained a good screen throughout the year.

Maintenance requirements were determined by supplying known weights of live minnow (*Phoxinus phoxinus*) to individual pike kept in four separate tanks. The weight of the fish was determined at intervals, when the amount of food was adjusted for the next period according to whether the pike had gained or lost weight.

For maximum growth pike were given known weights of minnow in excess of their consumption. At the end of approximately monthly periods the food fish remaining were removed and weighed. Three days later, in order to allow time for the digestion of any food in the stomach at the time of removal of the minnows, the pike were removed, weighed, and measured.

For levels of feeding below the maximum intake, pike were fed *ad libitum* for the first monthly period to establish a base line. Then they were given

gradually diminishing quantities of food until the level of the maintenance requirement was reached.

For the weighing and measuring process both food fish and pike were anaesthetised with tricane methanesulphonate (Sandoz M.S. 222) at a dilution of 1 in 15,000. After a few minutes in the anaesthetic the fish were removed, dried on either a filter paper or soft cloth, and weighed to the nearest .01 g on a Shandon balance. The length was determined by measuring from the tip of the snout to the fork of the tail. Large fish were measured to the nearest millimeter on a board, smaller ones by pricking onto waxed paper. The anaesthetic appeared to have little after-effect and pike would begin feeding within a few minutes of being reintroduced to their tank.

Tank temperatures were measured by means of a Six's maximum and minimum thermometer read three times weekly; from these recordings the mean weekly temperature was determined. Day and night fluctuations were relatively small and temperatures followed quite closely the surface temperature of Lake Windermere.

At the start of the work small pike were obtained by seining suitable areas of the lake, but in the spring of 1959 mature fish were taken and their eggs hatched artificially. These home-raised animals of known age formed the main experimental population and were all the progeny of one male and one female.

RESULTS

Maintenance

Preliminary investigations on maintenance requirement were carried out between March and August 1959 on six pike ranging in weight from 108 to 390 g. There was a marked increase in requirement from May to June followed by a subsequent decline (Fig. 1). It was apparent that there was very little difference between the maintenance requirement per gram of large fish and that of the smaller ones. In September of the same year

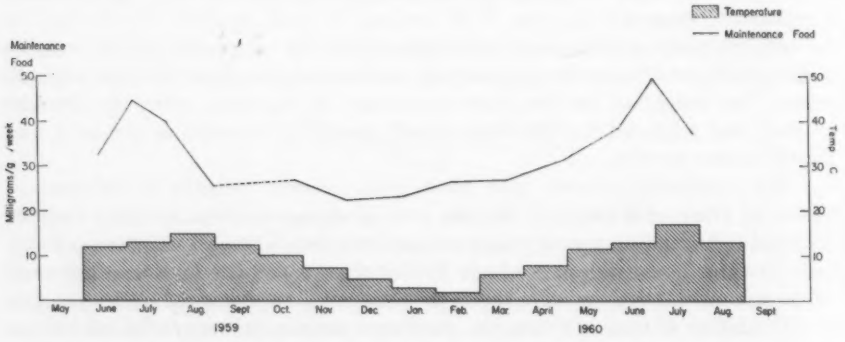

FIG. 1. The seasonal maintenance requirements of young pike.

these fish were replaced by four pike of known age, two being young-of-the-year and two a year older; their weights were 21, 29, 92, and 107 g. These fish were maintained close to their initial weight for 352 days without any visible deterioration in health. Figure 1 shows the mean maintenance requirement over the whole period from May 1959 to September 1960. Table I gives the amount of food consumed by the four pike of known age and also an adjustment for the slight gain in weight. The adjustment was made by converting the weight increase into food equivalent through the use of the efficiency of utilization of food at the maintenance level (ϵ_m) (see below), and subtracting this amount from the total consumption. The result was then corrected for a complete year.

The high value obtained for the fish F1 is due to an accident which occurred to it in June 1960 when it jumped from its own tank into that of a neighbour, getting badly wounded by the rightful occupant. During the recovery period, maintenance requirements increased from 45 to 67 mg per gram week. From the date of the accident the figures for F1 have not been included in computing the mean.

TABLE I. The determination of maintenance requirement September 1959 to September 1960.

	Pike no.				
	F_1	F_6	F_7	F_9	Mean
Initial wt (g)	29.25	91.5	21.30	107.0	
Final wt (g)	30.16	91.76	24.76	110.00	
Total food (g)	53.93	125.37	36.51	141.41	
Corrected food	52.09	125.11	29.31	135.11	
Annual maintenance (g/g/yr)	(1.847)	1.417	1.428	1.310	$1.385 \pm .053$

The uniformity of the results for fish F6, F7, and F9 is quite remarkable considering that there is a size difference of over 5 times between F7 and F9. There is no suggestion that the maintenance requirement is reduced with increasing size; even in the preliminary trial where one fish of 400 g was used there is no observable difference in requirement per gram.

Of great significance is the seasonal change in maintenance requirement which is apparently independent of temperature. The peak requirement occurs in June when 45 mg/g is necessary; this is followed by a rapid fall during July and August to a fairly stable level of 25 mg/g per week. Maintenance continues at about this level until February when a gradual increase is evident; this accelerates during May until it reaches the June peak. The mean weekly maintenance ration is 26.7 mg/g with a yearly total of $1.385 \pm .053$ g/g which is the *annual maintenance coefficient* (ζ).

Since there is no discernible change in maintenance requirement with increasing size (i.e. $j = 1$) the maintenance equation may be written:

$$\frac{d\xi_m}{dt} = \zeta w \qquad \ldots\ldots (i)$$

GROWTH

Running concurrently with the observations on maintenance requirements were observations on growth. Five tanks were employed, each containing a single pike fed known weights of minnow in excess of their needs. Regular determinations of increase in weight and length and food consumed were made from March 1959 until May 1960. At this point a rearrangement was carried out so that two fish continued on full rations whilst a further six were fed on a diminishing scale, with the object of obtaining information on the efficiency of utilization at levels below that of maximum consumption. The term "efficiency of utilization" is used in the same sense as Beverton and Holt who discuss the usage of the term more fully; the meaning is: grams of pike produced per gram of food.

The annual cycle of growth is shown in Fig. 2; the growth at approximately monthly intervals is expressed as the specific growth rate per cent, per unit time. The general pattern of growth is very similar to that of the maintenance requirement, in that it increases gradually from February, reaches a peak in late June, followed by a fall through July, and subsequently maintains a relatively steady state until a decrease occurs in December. During February, when tank temperatures fell close to zero, some fish actually lost weight although there was no comparable loss in those fish held on a maintenance ration.

At the close of the experiment the two pike F2 and F3 which had been allowed to run the whole year on full rations, were killed; both were found to be females; F2 was completely immature whereas F3 had gonads at the earliest stage of development. F3 showed a slightly higher growth rate during July and August than did the immature one. A summary of the results for these two fish is presented in Table II.

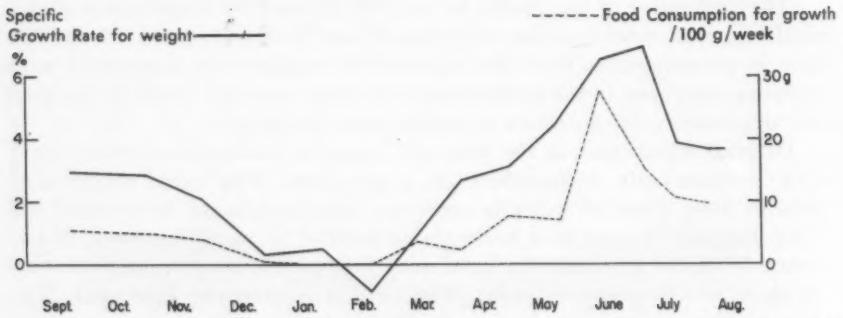

FIG. 2. The specific growth rate per cent per unit time of young pike for the period September 1959 to September 1960.

TABLE II. The food and growth of young pike for the period September 1959 to September 1960, and the determination of the efficiency of utilization (ϵ).

	Pike no.		
	F_1	F_2	Mean
Initial wt (g)	39.10	39.60	
Final wt (g)	145.54	154.55	
Increment (g)	106.44	114.95	
Total food (g)	336.07	393.35	
Mtce. food (g)	107.88	111.79	
Food for growth (g)	228.19	281.56	
$\epsilon = \frac{\text{Increment}}{\text{Food for growth}}$	.466	.408	.437
Gross conversion $= \frac{\text{total food}}{\text{increment}}$	3.16	3.45	3.31

Data obtained from the pike fed on a diminishing scale are shown in Table III. No consistent trends were observed in the efficiency of conversion as the rations diminished, although there was a considerable variation in the efficiencies recorded. The maximum value for each pike was extracted and a mean result of $0.485 \pm .033$ was obtained. This is the *maximum efficiency of utilization* (ϵ_0) (Table III).

DERIVED RESULTS

From the above data on the maintenance requirements and growth of pike it is possible to determine the efficiency of utilization at various levels of feeding and the maintenance energy coefficient (Q).

The overall efficiency of utilization (ϵ) is obtained by subtracting the calculated maintenance requirement from the total food of the pike maintained

TABLE III. The determination of the maximum value of the efficiency of utilization (ϵ_0).

	Pike no.						
	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	Mean
Maximum value of efficiency of utilization (ϵ_0)	.587	.517	.467	.486	.450	.403	$.485 \pm .033$

on full rations throughout the year, and dividing the remainder into the increase in weight; this gives the grams of pike produced per gram of food. By this method a mean value for ϵ of 0.437 is obtained (Table II).

The efficiency of utilization at the maintenance level of feeding (ϵ_m) is determined from the equation:

$$\epsilon_m = \frac{1}{k\zeta\bar{w}^j} \tanh (k\epsilon_0 \zeta\bar{w}^j).$$

The only unknown is k, the reciprocal of the maximum rate of assimilation of energy. $1/k$ is obtained from the equation due to Kostitzin (Beverton and Holt, 1957):

$$1/k = \frac{\Delta_w/\Delta_t}{\tanh (k\epsilon_0 \Delta\xi/\Delta_t) - \tanh (k\epsilon_0 \zeta\bar{w}^j)}$$

where:

Δ_w/Δ_t = increase in weight per unit time (one year),

$\Delta\xi/\Delta_t$ = food consumption per unit time (one year).

Owing to the form of this equation it is not possible to solve directly for k and a process of successive approximations is necessary. The values obtained by this method are given in Table IV, along with the equivalent values of ϵ_m, which has a mean of 0.476.

From the efficiency of utilization at the maintenance level and the maintenance food coefficient the maintenance energy coefficient (Q) can be obtained:

TABLE IV. Values of the reciprocal of the maximum rate of assimilation of net energy by an individual fish (k) and values of the efficiency of utilization at the maintenance level (ϵ_m).

	F_2		F_3		
Date	k	ϵ_m	k	ϵ_m	Mean ϵ_m
May 10					
May 29			.0035	.481	
June 25	.0013	.485	.0017	.482	
July 11	.0020	.481	.0017	.477	
July 26	.0027	.447	.0028	.479	
Aug. 13	.0021	.480	.0017	.477	
Mean:		.473		.479	.476

$$Q = \epsilon_m \times \zeta$$

$$= 0.476 \times 1.385$$

$$= 0.659.$$

DISCUSSION

The determination of maintenance requirements over the period of a full year brings to light the significant fact that this is not a constant factor dependent upon temperature. There is apparently a significant change in metabolism during the late spring when the actual requirement nearly doubles. Although temperature undoubtedly does have a significant effect it can be overridden by this seasonal factor. It is highly probable that this factor is stimulated by increasing daylength, the peak requirement being reached at the time of the summer solstice. Swift (1955) found that at this season of the year brown trout (*Salmo trutta*) experienced a period of maximum thyroid gland activity indicating that considerable metabolic change is taking place.

Previous estimates of maintenance requirement must be used cautiously because they have not generally taken this seasonal effect into account. Dawes (1930, 1931) carried out a large number of feeding experiments on young plaice (*Pleuronectes platessa*) and from these data Beverton and Holt derived a figure of 20.48 g *Mytilus* flesh per gram of plaice per annum for the maintenance coefficient. This is greatly in excess of the figure of 1.39 g obtained for pike. Brown (1946a, b) determined the maintenance requirement of brown trout and found that it diminished over the first month as the fish adjusted to a low food intake. Subsequently, there was a nonlinear variation with temperature: between 5 and 9 C there was a gradual increase followed by a more rapid rise to 11 C; after this there was a second gradual rise to 20 C; above this temperature the maintenance requirement did not increase. These differences were attributed largely to variation in activity with temperature. Probably one of the reasons for the uniformity of the results in the pike is that activity remained at a very low level throughout the experiment; the only noticeable movement was when the fish were actually feeding.

Brown also showed for brown trout that the maintenance requirement tended to decrease with increasing size; at a standard temperature of 11.5 C it diminished as the fish grew larger to a constant level of about 60 mg of meat per gram of fish per week. This would give a maintenance coefficient of 3.12 g on an annual basis, which is comparable to the figure obtained for pike when account is taken of the higher temperature at which the determination on trout was made. Beverton and Holt, again using Dawes' data, concluded that the maintenance requirement decreased as the 2/3 power of the weight in plaice. This, decrease is not apparent in pike and again may be connected with the very low level of activity observed.

Scholz (1932) determined the requirement of young pike at a temperature of 18–19 C and concluded that 1–2% of the body weight in food was required daily, which would mean a maintenance coefficient between 3.7 and 7.3 g per gram per annum. Considering the high temperature of the trial these figures seem to be fairly well in line with the present results.

Growth follows food consumption very closely and reaches a peak at the time of maximum maintenance requirement in late June. The pike maintained for a year on full rations achieved a growth rate comparable to wild fish at the same stage. Frost and Kipling (1959) calculated the age and growth of pike in Windermere: medium-paced growers reached 20 cm at the end of the first year of life and 36 cm at the end of the second. At the age of 1 year the experimental fish reached 21.0 cm and had achieved 27.8 and 28.3 cm at the end of 18 months, which is comparable with the wild fish of the medium-paced group. Thus, it seems that the tank environment has not had a deleterious effect on the growth pattern.

After maintenance requirements have been met in full, the balance of the food is converted at the rate of 1 g food to .437 g pike. This is often expressed as the "net conversion" which is 2.28 g food per gram pike produced. When the maintenance requirement is included the "gross conversion" is obtained which for the experimental pike is 3.41. Brown trout, which have been intensively studied give gross conversion rates of 2.3–7.1 (Brown, 1957); Beverton and Holt give a net figure of 5 to 1 for plaice, and Moore (1941) derives a net value between 2.3 and 3.9 for the green sunfish (*Apomotis cyanellus*).

Pike, therefore, seems to be outstanding amongst those species studied, for its maintenance requirement and the high rate at which it converts food to body-substance. However, as will be shown in a second paper (Johnson, 1966) it is the maintenance food which has the greatest significance in determining the total food consumption, and this is dependent to a great extent on the size of the individuals in the population.

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Muscular Fatigue and Mortality in Haddock, *Melanogrammus aeglefinus*, caught by Otter Trawl[1]

By F. W. H. Beamish[2]

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

ABSTRACT

Haddock were subjected to strenuous muscular exertion in capture by otter trawl. In each of seven tows, blood lactic acid followed a similar pattern with peak levels between 90 and 240 min after capture. Generally, in surviving fish, lactic acid subsided to preexercise levels within 12 hr after capture. Tagging fish immediately after capture had no demonstrable influence on lactic acid level during recovery. Among the seven tows, mortality associated with fatigue ranged from 0 to 27%. Mortality estimates were made for an additional nine tows. Where mortality was attributable to fatigue, lactic acid varied between 100 and 220 mg/100 ml, with a mean of 150 mg/100 ml. Percentage mortality ranged between 7 and 78.

INTRODUCTION

As early as 1938, von Buddenbrock suggested that Atlantic cod, *Gadus morhua*, may die following severe muscular exertion. Fatigue deaths among tench, *Tinca tinca*, after 15 min strenuous exercise were observed by Secondat and Diaz (1942). More recently, mortalities attributable to muscular fatigue were reported for several of the Salmonidae: chinook, *Oncorhynchus tshawytscha* (Parker and Black, 1959); coho, *O. kisutch* (Parker et al., 1959; Milne and Ball, 1956); sockeye, *O. nerka* (Black, 1957c). Fatigue deaths among yellowfin tuna, *Neothunnus macropterus*, and skipjack, *Katsuwonus pelamis*, were observed by Barrett and Connor (1962) following capture by hook and line. In contrast, instances of strenuous exercise with no ensuing mortality have been reported for a number of species. Among the Salmonidae, rainbow trout, *Salmo gairdneri* (Black, 1957a), lake trout, *Salvelinus namaycush* (Black, 1957b), coho and chinook salmon (Paulik et al., 1957; Jensen, 1958, in Parker et al., 1959) suffered no mortality after severe exercise. In each case where mortality did not occur, fish were starved prior to strenuous exercise in fresh water. The instances where fatigued salmon died are, in contrast, in the saltwater environment, and usually involve fatigue in capturing.

The exact cause of death is unknown (Black, 1958). However, in instances of delayed mortality among injury-free fish, blood lactic acid is particularly high. Indeed, Parker and Black (1959) demonstrated a significant correlation between death and lactic acid for chinook salmon. Many investigators have used lactic acid as a yardstick of fatigue following exercise.

[1]Received for publication May 6, 1966.
[2]Present address: Department of Zoology, University of Guelph, Guelph, Ontario.

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In the Northwest Atlantic, groundfish are frequently captured by otter trawl for tagging purposes. The gear is towed along bottom at speeds between 140 and 200 cm/sec for approximately 30 min. Underwater television observations suggest fish swim ahead of the footrope for some time before either falling back into the net, or, in the case of small fish, escaping through meshes. Of the frequently tagged species, haddock, *Melanogrammus aeglefinus*, is particularly suitable for fatigue studies. That mortalities occur among haddock following strong exercise is suggested by McCracken's (1956) observations on tag recoveries. He found greater tagging mortalities among haddock than Atlantic cod when both were captured in the same manner and subjected thereafter to similar fishing intensity. That fatigue death is an important consideration in tagging studies is supported by Parker et al. (1963). However, no information is available on fatigue and mortality in fish after capture by otter trawl.

MATERIALS AND METHODS

All tows were made by the research vessel M.B. *Mallotus*, equipped with a small otter trawl having a footrope of about 16 m and a headline height of 2 m. The gear was towed along the seabed at 140 cm/sec for 30 min. All tows were made between May and August at depths from 45 to 55 m. During this period bottom temperatures increased from 4.8 to 9.5 C.

Immediately after the net was brought aboard at the end of a tow, haddock of similar size (and judged to be free of injury) were placed in live holding tanks. Haddock displayed no obvious signs of nervous excitement in live tanks. Activity was low for some time after they were first put in a tank. Afterwards it increased but was never excessive. Two sizes of holding tanks were used. The larger was approximately 180 cm in diameter and 120 cm deep; the smaller was 90 cm in diameter and 60 cm deep. Generally, fewer than 50 haddock (40 cm) were kept in the larger tank and 30 in the smaller. Tanks were supplied with sufficient flows of water to maintain oxygen at or near air saturation. Temperatures in the holding tanks ranged from 1.3 to 2.7 C degrees higher than the bottom temperature. On completion of one or two tows, the research vessel returned to the laboratory. This took about 1 hr.

Catches were handled in either of two ways. In one case, blood samples were taken at regular intervals of 0, 60, 90, or 150, 240, 480, and 720 min after capture and lactic acid concentration measured. Usually 10 fish were taken for each sample except for the 60-min recovery period when five were sampled. Each fish was used once. Blood samples were not taken from those fish that died during 12 hr recovery; however, time to death was recorded. Each fish was sampled for length (fork), weight, sex, otoliths, and volume of stomach contents. Age was determined from the otoliths.

In a second group of experiments, blood samples were taken only from fish that died during recovery. Samples were taken immediately prior to death. This time was judged from respiratory activity. As death approached,

respiratory movements became less regular and shortly before death the interval lengthened noticeably. Often convulsive movements accompanied terminal stages. Fish were sampled for length, weight, sex, otoliths, and volume of stomach contents after blood samples were taken. Observations were continued for 12 hr from the end of a tow. Whenever practicable at the end of an experiment, blood lactic acid was measured for 10 haddock to establish the level of recovery.

Unexercised levels of blood lactic acid were measured in the laboratory. Fish were captured by otter trawl and brought to the laboratory where they were transferred to holding tanks. Tanks were approximately 90 cm in diameter and 60 cm deep. Temperature was 9.5 C and within 1.5 C degrees of that where haddock were captured. Fish were held for 3 days before being netted from the tanks and sampled for blood lactic acid.

Blood samples were taken by cardiac puncture with a heparinized 2-ml syringe lubricated with paraffin oil. Cold 10% trichloracetic acid was used to deprotenize the blood. The mixture was filtered and the filtrate processed according to the method of Barker and Summerson (1941). Filtrates of all samples taken at sea were stored in ice until the vessel's return when the analyses were completed as quickly as possible. Those samples taken after the vessel's return were analysed immediately. Lactic acid concentrations were determined in duplicate and are expressed as milligrams of lactic acid per 100 ml of blood (mg/100 ml).

Standard error of the mean, as outlined in Stanley (1960), was determined for lactic acid concentration and body length. Analyses of variance were applied to blood lactic acid within each recovery period among the seven tows (Snedecor, 1948). Square root transformations and, in one case, log transformation, were used to reduce variance. Uniformity of variance was estimated from the test given in Owen (1962). Mortality was calculated in either of two ways. Where deaths occurred between recovery sampling periods, mortality estimates required adjustment for the regular reduction of stock. For each postexercise interval, mortality was calculated as $1-s$ where s is survival. This was accumulated with preceding intervals as $1-s_1, s_2, s_3 \ldots . s_n$. To express mortality in per cent, the result was multiplied by 100. For those tows where only mortality was followed, the number of deaths was calculated as simple percentage of total. Significant differences in mortality between males and females were examined by chi-square test for 2 by 2 table (Snedecor, 1948).

Ideally, after capture, haddock would have been tagged and placed in live tanks. However, it was not practical to have a technician trained in fish tagging accompany each trip. Hence a comparison was made of blood lactic acid levels between tagged and untagged fish. Following capture by otter trawl, one group of 10 was placed directly in a live tank. A second group of 10, from the same catch, was first tagged and then placed in the same holding tank. After 60 min recovery blood lactic acid was determined for the two groups. For tagged fish, mean lactic acid was 86.5 mg/100 ml with a standard

error of 11.7, and for untagged, 82.3 ± 9.3 mg/100 ml. All fish were 3 years old. Obviously there was no significant difference between the two groups. Thereafter haddock were not tagged.

RESULTS

Concentration of blood lactic acid in unexercised fish was 5.9 ± 1.2 mg/100 ml for 10 two-year-old fish. Mean length and weight, along with standard errors, were 36.0 ± 1.5 cm and 445 ± 17 g, respectively.

Postexercise change in blood lactic acid was determined for seven tows (Fig. 1 and Appendix I). Haddock were of similar size (Appendix I) and age (3-year-olds). At time of capture all fish contained food in their stomachs. The pattern describing postexercise changes in lactic acid was similar for each tow. Immediately after a catch was released from the net (0 min recovery), lactic acid was approximately 7–10 times higher than that in the unexercised

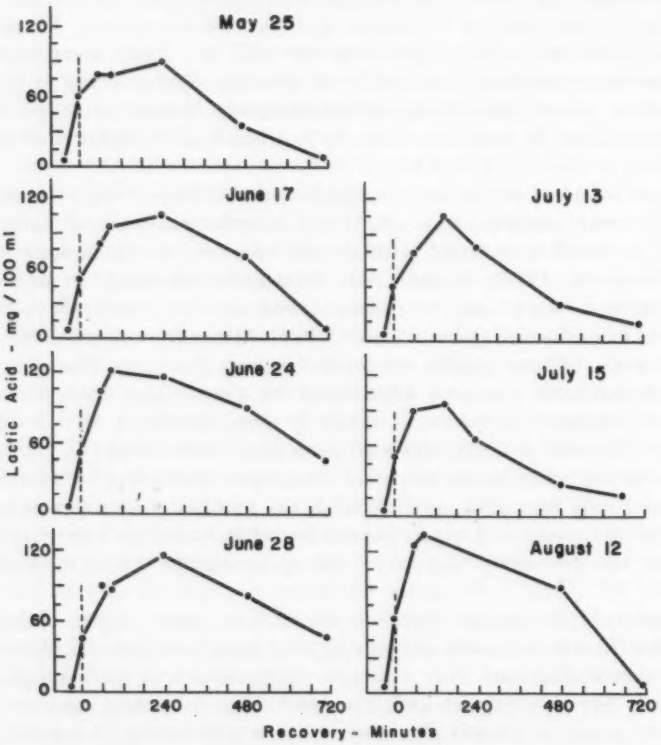

FIG. 1. Blood lactic acid in haddock following capture by otter trawl. For each tow, a vertical broken line is drawn through mean lactic acid concentration immediately after capture (0-min recovery). Preexercise lactic acid was determined in the laboratory.

state. With further recovery lactic acid increased, reaching a peak between 90 and 240 min. Peak values were about twice those at 0 min recovery. From the peak value, lactic acid diminished gradually with increase in recovery until after 720 min it usually approached unexercised levels. However, in two tows, June 24 and 28, after 720 min, lactic acid remained high and about equal to levels at 0 min recovery.

While patterns of recovery were similar, lactic acid concentration varied among tows for comparable recovery. Variance among tows was significant at the 5% level for each recovery represented by more than two tows (Table I). Generally, within each recovery period of each tow, variance was uniform after data were subjected to square root or log transformations. Uniformity was not observed in three instances: May 25, 0 and 720 min recovery; June 24, 720 min recovery. These groups were not included in calculations. Although not significant in all cases, some tows had higher concentrations of lactic acid than others over most recoveries. Thus, tows made on June 24, 28, and August 12, generally had the highest concentrations of acid for comparable recovery. There was no demonstrable influence of temperature on blood lactic acid over the range 6.8–12.0 C (May 25 to August 12).

Some postexercise mortality was observed during each tow (Appendix I). In one tow (June 17) only one death was observed from an initial stock of 71 whereas, in another (June 24), 31 of 100 died during 720 min recovery. After adjustment for regular reduction of stock, mortality estimates ranged from 2 to 42%. The majority of deaths occurred during the first hour (Appendix I).

TABLE I. Summary of analyses of variance within each recovery among seven tows. Square-root transformations were used in all cases except for recovery of 720 min where data were subjected to log transformation.

	Mean square					
Recovery	Individuals	df	Tows	df	F	Significance
(*min*)						(%)
0[a]	0.733	53	2.547	5	3.475	1
60	0.661	28	4.027	6	6.092	1
90	2.168	45	13.100	4	6.060	1
150	1.366	18	2.157	1	1.579	5
240	6.441	53	16.193	5	2.514	5
480	9.449	58	30.229	6	3.199	1
720[b]	0.308	45	5.545	4	18.003	1

[a]Tow made on May 25 was omitted.
[b]Tows made on May 25 and June 24 were omitted.

Indeed, for two tows (June 17 and July 13) all mortalities took place during the first 60 min after exercise. In the other tows, 45–82% of total mortality occurred during the first hour. Between 60 and 90 min, deaths were observed in only one tow. Some mortality occurred thereafter, but in all cases it was considerably less than that during the first 60 min.

There was some indication for those tows with high mortality that lactic acid levels during recovery were correspondingly large. Correlation between mortality and peak lactic acid, using the Kendall rank correlation method (Siegel, 1956), was significant (T = 0.71).

Deaths among male and female haddock were compared for each tow with numbers surviving after 720 min by a chi-square test. Significant differences were not observed ($P > 0.05$) except in the tow made on July 13 where mortality of males was greater than that of females ($P < 0.01$). When results of all tows were pooled, differences were not significant ($P > 0.20$).

Nine tows were made in which sampling was confined to postexercise deaths. Mortalities were separated according to time of death into one of eight postexercise intervals (Table II). Few deaths were observed during the first 90 min after capture. Mortality increased sharply between 90 and 239 min and remained high to 479 min. Deaths declined thereafter, with few between 480 and 659 min. None were observed between 660 and 720 min. Percentage mortality ranged between 12 and 85.

TABLE II. Mortality following capture in a 30-min tow.

Date	Tow no.	Tank temp	Total used	Total deaths	Length ± SE	Number of deaths postexercise (*min*)							
						0–14	15–29	30–59	60–89	90–239	240–479	480–659	660–720
		(*C*)	(*no.*)	(*no.*)	(*cm*)								
Aug. 16	1	12.0	49	15	38.1±1.3	–	–	–	1	5	8	1	–
Aug. 16	2	12.0	36	9	38.0±2.1	–	–	–	–	3	6	–	–
Aug. 12	1	12.6	61	7	37.1±2.2	1	–	1	–	2	2	1	–
Aug. 12	2	12.6	24	5	33.2±0.9	–	1	–	–	–	4	–	–
Aug. 10	1	12.0	78	11	34.7±0.8	2	1	2	–	2	1	3	–
Aug. 10	2	12.0	29	8	36.2±1.2	–	–	–	–	4	2	2	–
Aug. 4	1	12.0	56	46	38.3±1.0	1	–	2	–	13	26	4	–
Aug. 2	1	12.0	29	4	39.7±0.8	2	–	–	–	1	–	1	–
Aug. 2	2	12.0	36	15	40.8±1.4	–	2	1	–	10	2	–	–

Blood lactic acid for fish dying within 60 min after exercise generally was less than that thereafter. During the first 14 min after capture, mean blood lactic acid was 58.0±6.4 mg/100 ml; between 15 and 29 min, 67.8±5.6 mg/100 ml; between 30 and 59 min, 83.3±9.3 mg/100 ml. Hence, among early mortalities, blood lactic acid approximated that for surviving fish after comparable recovery. After 60 min, blood lactic acid increased notably. Indeed, the mean of 70 samples collected between 60 and 660 min was 149.6 mg/100 ml, with an SE of 4.9. Individual concentrations varied between 100 and 220 mg/100 ml.

The number of male and female mortalities in relation to those surviving was compared. No significant difference was found ($P > 0.05$) in eight of the nine tows. In one tow (August 4), a significantly greater number of females died than males ($P < 0.01$). When all tows were grouped, significant differences were not found ($P > 0.20$). Blood lactic acid of survivors after 720 min approached unexercised concentrations (Table III).

TABLE III. Blood lactic acid concentrations in haddock after 720 min recovery. For each tow, 10 fish were sampled.

Date	Tow no.	Length±SE	Weight	Blood lactic acid±SE
		(cm)	*(g)*	*(mg/100 ml)*
Aug. 16	1	40.8±1.5	743	8.6±2.0
Aug. 16	2	35.6±1.1	415	4.8±1.1
Aug. 12	1	35.4±0.5	442	24.8±7.3
Aug. 12	2	34.5±1.1	383	14.5±1.9
Aug. 10	1	36.1±1.1	532	9.0±1.8
Aug. 10	2	32.8±0.7	369	3.6±1.2

DISCUSSION

After capture by otter trawl, blood lactic acid in haddock followed the same pattern as that found for other species subsequent to strong exercise (Table IV). Relatively low concentrations increased with exercise, reaching peak accumulations in the case of haddock, 90–240 min afterwards. Thereafter, blood lactic acid decreased to about unexercised levels some 12 hr later. At this time it was presumed recovery was complete.

Suggestions for the postexercise delay in disappearance of lactic acid are discussed in Black et al. (1961). Based on work by Johnson et al. (1945), they suggested diffusion is comparatively slow at low temperatures. In accord, Barrett and Connor (1962) found lactic acid in tuna disappeared much faster following exercise at 26–29 C than that in other species at low temperatures.

TABLE IV. Blood lactic acid levels for various species before and after strenuous exercise.

Investigator	Species	Age	Exercise period	Temp	Unexercised lactate	Lactate after exercise	Peak lactate	Time to peak lactate	Time to unexercised lactate levels	Fresh or salt water
		(yr)	*(min)*	*(C)*	*(mg/100 ml)*	*(mg/100 ml)*	*(mg/100 ml)*	*(hr)*	*(hr)*	
Secondat and Diaz (1942)	Tench, *Tinca tinca*	–	15		16	53	–	–	–	fresh
Black (1955)	Sucker, *Catostomus catostomus*	1	15	11.5	17	55	–	–	–	fresh
	Carp, *Cyprinus carpio* (1953)	3	15	11.5	9	54	–	–	–	fresh
	(1954)	3	15	11.5	9	78	–	–	–	fresh
	Bass, *Micropterus salmoides*	1½	15	11.5	17	76	–	–	–	fresh
	Catfish, *Ameiurus melas*	2½	15	11.5	8	35	–	–	–	fresh
	Squawfish, *Ptychocheilus oregonensis*		15	11.5	19	94	–	–	–	fresh
Black (1957a)	Rainbow trout, *Salmo gairdneri*	1	15	11.5	16	100	170	1	16	fresh
		2	15	11.5	9	82	120–140	1–4	16–18	fresh
Black (1957b)	Lake trout, *Salvelinus namaycush*	1½	15	11.5–12.0	14	92	177	2	24	fresh
Black (1957c)	Sockeye salmon, *Oncorhynchus nerka*	1	15	8–12	17	112	–	–	–	fresh
		1	15	8–12	24	167	–	–	–	salt
		2	15	20	20	103	278	2½	8	salt
Miller et al. (1959)	Rainbow trout	1½	15	4.4–11.7	11	45	–	–	–	fresh
Parker and Black (1959)	Chinook salnom, *Oncorhynchus tshawytscha*	immature	3–30	14–15	–	65	196	3½–4		

Parker et al. (1959)	Coho salmon, *O. kisutch*	adult	1–30	–	20	38	40	1½	–	fresh
Black et al. (1960)	Rainbow trout	1½	15	11.5	8	49	96	2	12	fresh
Heath and Pritchard (1962)	Bluegill sunfish, *Lepomis macrochirus*	–	11	20	10	87	100	1	10	fresh
Barrett and Connor (1962)	Yellowfin tuna, *Neothunnus macropterus*	–	0.7	26–29	–	14–23	115	½	–	salt
	Skipjack tuna, *Katsuwonus pelamis*	–	0.7	26–29	–	14–23	160	¾	–	salt
Barrett and Connor (1964)	Yellowfin tuna, *Thunnus albacares* (tagged)	–	.07–.20	17–23	–	6.0	82	¾–1	–	salt
	Yellowfin tuna (untagged)	–	.07–.20	17–23	–	5.4	60	1–1½	–	salt
	Skipjack tuna (tagged)	–	.07–.20	17–23	–	10.3	161	¾–1	–	salt
	Skipjack tuna (untagged)	–	.07–.20	17–23	–	13.0	150	¾–1	–	salt
Caillouet (1964a)	Carp (winter)	adult	15	1	9	22	22	0	4½	fresh
	(summer)	adult	15	24	14	29	29	0	2–2½	fresh
Caillouet (1964b)	Channel catfish,	–	5	14–17.7	2–8	21–44	–	–	–	fresh
	Ictalurus punctatus	–	15	14–17.7	2–8	59–68	–	–	–	fresh
Wendt (1965)	Atlantic salmon, *Salmo salar* (winter)	2	32	0.2	3.5	38.8	57	2	>15	
	(summer)	1½	21	15	14.6	76.3	175	2	15	

Circulation may be impaired by influence of lactic acid on the heart. Redfield and Medearis (1926) demonstrated a reduction in activity of turtle heart in the presence of lactic acid. Finally, the large quantity of acid produced could account for a lag in its elimination (Black et al., 1961).

Comparison of absolute levels of lactic acid among different species suffers in that duration of exercise often is not standard. For example, Heath and Pritchard (1962) exercised bluegill sunfish, *Lepomis macrochirus*, for 5–45 min, while Black (1957a, b, c) and others maintained a 15-min period in laboratory studies. Where exercise consisted of capture on commercial gear, large variations occurred. Thus, coho salmon were landed between 1 and 30 min after hooking (Parker et al., 1959). Barrett and Connor (1962) found average time from hooking tuna to release in live tanks was 40 sec. More recently, Barrett and Connor (1964) reduced the time to about 6 sec. Similarly, in the present study, capture of haddock could have occurred at any time during the 30-min tow. It is also hard to assign qualitative figures to strength of exercise and physical condition of fish. Unquestionably, species variation in blood circulation plays a significant role in the rate of lactic acid accumulation and disappearance. Nevertheless, there is striking similarity in pattern of lactic acid following strong exercise. Blood lactic acid was low among all species prior to exercise. For haddock, acid was 6 mg/100 ml and within the range, 2–24 mg/100 ml, reported for other species (Table IV). Immediately after exercise, lactic acid increased among the various species from two to about thirteenfold. In haddock, levels rose between six and eleven times. Further, almost one-half of the species had lactic acid concentrations within the mean range found for haddock (45–63 mg/100 ml). Recovery to maximum acid levels varied considerably among species as did absolute concentration. A peak concentration of 22 mg/100 ml was found in carp by Caillouet (MS, 1964b) immediately after exercise. In contrast, chinook salmon reached maximum levels of 196 mg/100 ml between 210 and 240 min after capture (Parker and Black, 1959). Peak concentrations of lactic acid in haddock occurred between 90 and 240 min after exercise. That lactic acid returned to unexercised levels for most species within 8–16 hr after exercise is concordant with observations on haddock.

Considerable variation was found in blood lactic acid in haddock at death. Among mortalities, there was a notable separation in lactic acid concentration before and after 60 postexercise min. After 60 min, acid levels ranged from 100 to 220 mg/100 ml with an overall mean of 150 mg/100 ml. This was in contrast to the lower levels found for mortalities within 60 min. Generally, acid levels in early deaths followed closely those of recovering fish. Hence, it was presumed early mortalities were primarily associated with causes other than fatigue. This would include unrecognized mechanical injury suffered in the net. For the most part, blood lactic acid among mortalities after 60 min was appreciably higher than that found after comparable periods of recovery. In these cases, mortalities were attributed primarily to muscular fatigue. This does not preclude the possibility of contributory effects from other agents.

That most fatigue deaths are delayed in occurrence was demonstrated for troll-caught coho salmon by Parker et al. (1959). They observed most mortalities between 1 and 3 hr after capture. In the present study, relatively large numbers of deaths were observed up to 8 hr after exercise.

Critical threshold of lactic acid in haddock is regarded as 100 mg/100 ml. Of course, this is not to suggest all haddock with lactic acid levels in excess of 100 mg/100 ml will die. Clearly it is not the case. Among fish sampled during recovery, many had acid concentrations of 100 mg/100 ml. Indeed, the overall mean of 50 fish sampled 90 min after capture was 103 mg/100 ml. This is perhaps high as there was no evidence that all sampled fish would have recovered had they not been killed.

Blood lactic acid concentration at death has been measured for three salmonids (sockeye, coho, and chinook). All demonstrated wide variations in acid concentration. Parker et al. (1959) found a range of 123–318 mg/100 ml, and a mean of 200 mg/100 ml for troll-caught coho salmon. They estimated the danger zone in coho to be above 125 mg/100 ml. In troll-caught chinook salmon, lactic acid ranged between 108 and 226 mg/100 ml, with a mean of 189 mg/100 ml (Parker and Black, 1959). Among sockeye mortalities, Black (1957c) obtained lactic acid levels between 208 and 292 mg/100 ml, with a mean of 240 mg/100 ml.

On the assumption that early haddock deaths were not directly associated with fatigue, mortality estimates ranged between 0 and 78%. For the nine tows where simple percentage mortality was calculated, estimates varied between 7 and 78%. Corrected adjusted cumulative mortality in the other seven tows ranged from 0 to 27%. Variation in mortality may relate to differences in physical condition. Measurements of glycogen were not made; nor was it possible to estimate activity prior to capture.

No information on mortality following capture by otter trawl was found in the literature. However, estimates of mortality have been made on fish strenuously exercised in other ways. Fatigue deaths in ocean troll-caught chinook and coho salmon were observed by Milne and Ball (1958), Parker and Black (1959), and Parker et al. (1959). Mean estimates varied between 18 and 71%. At least part of the variation was attributed to differences in length of recovery period. Black (1957c) found 25% mortality among sockeye salmon following 15 min chasing in a hatchery trough. After a similar treatment, Secondat and Diaz (1942) observed 20% mortality among tench. Litt (1954, in Black, 1958) reported 25% mortality among striped bass, *Roccus saxatilis*, swimming to avoid a fish screen. Barrett and Connor (1962) attributed some of the deaths of line-caught yellowfin and skipjack during recovery to fatigue but were unable to estimate percentage.

Actual cause of death is unknown. In his comprehensive review on hyperactivity, Black (1958) discusses probable reasons for fatigue death. These include interference with the acid-base equilibrium coupled with, in the presence of excess acid, reduced affinity of hemoglobin for oxygen and, in the presence of excess alkali, reduced affinity for carbon dioxide. Recent

studies on carp metabolism by Wittenberger and Diaciuc (1965) support the suggestion of inadequate oxygenation as a cause of death. Depletion of energy reserves may also play a significant role in death of fish. That haddock contained food in their stomachs when captured certainly would not enhance the probability of survival. With fish in debt from severe muscular exertion, the additional oxygen demand associated with digestion may in some cases add sufficient stress to cause death.

For tagging purposes it may be profitable to hold haddock in live tanks for the critical mortality period. Increased tag returns in coho salmon were found for fish held in live tanks not less than 1 hr after tagging. It is probably beneficial to tag fish immediately after capture and well in advance of release. At this time stress of tagging has less effect. After 60 min recovery, no significant difference in blood lactic acid was observed between untagged haddock and those tagged immediately after capture. Delayed tagging may increase mortality. Fry and Hughes (1951) found low recoveries among chinook salmon held overnight in live tanks before tagging and release.

A second approach is to tag fish immediately after capture, releasing some and holding others. Deaths among those held might be used to estimate mortality for those released.

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APPENDIX I. Blood lactic acid in haddock captured in 30-min tows and allowed various periods of recovery. Standard errors are included for mean length and lactic acid concentration.

Date	Holding temp	Recovery	Weight	Length ± SE	Stomach contents	Lactic acid ± SE	Deaths	Accumulated adj. mortality
	(*C*)	(*min*)	(*g*)	(*cm*)	(*cc*)	(*mg/100 ml*)	(*no.*)	(%)
May 25, 1965	6.8	0	742	40.7±1.3	6.2	63.0± 8.9	–	–
		60[a]	662	40.0±1.4	2.7	85.3± 4.8	8	14
		90	759	40.3±1.8	3.1	81.1± 8.5	–	14
		240	583	37.0±1.9	1.8	90.1±23.6	–	14
		480	698	39.7±0.6	3.6	36.4± 8.9	1	17
		720[a]	661	38.2±1.0	3.4	5.8± 2.6	–	17
June 17, 1965	10.0	0	784	40.9±1.4	2.2	46.7± 5.1	–	–
		60[a]	836	42.4±1.2	2.0	81.6± 7.2	1	2
		90	819	41.4±1.2	2.0	94.0± 7.9	–	2
		240	794	43.6±1.6	1.5	107.3±10.9	–	2
		480	862	42.5±2.2	2.8	69.8±19.8	–	2
		720	750	41.1±1.8	1.5	4.6± 0.8	–	2
June 24, 1965	9.5	0	972	43.6±1.2	2.0	54.6± 3.9	–	2
		60[a]	929	43.0±2.1	1.6	103.4± 9.7	19	21
		90	950	43.8±0.9	2.6	120.7±10.0	–	21
		240	1037	46.0±0.6	1.8	116.4±13.8	6	30
		480[a]	949	43.9±1.2	2.4	90.7±19.7	4	37
		720	1023	45.5±1.0	2.0	41.5±15.4	2	42

June 28, 1965	9.6	0	1044	45.5±1.2	2.2	44.5± 7.4	–	–
		60[a]	1081	44.4±1.4	2.6	88.7± 3.9	18	22
		90	1038	45.4±1.1	2.4	86.6± 7.6	–	22
		240	994	44.4±1.1	3.4	115.6±11.1	2	25
		480	1200	47.1±0.9	2.3	82.1± 5.7	–	25
		720	995	44.1±1.6	2.8	44.5± 8.6	3	33
July 13, 1965	9.8	0	641	38.1±1.4	1.4	45.1± 2.9	–	–
		60[a]	612	37.3±1.1	1.1	83.1± 4.7	12	18
		150	835	41.0±0.9	2.0	107.8± 6.6	–	18
		240	707	38.9±1.7	1.4	79.5±13.7	–	18
		480	873	42.1±2.4	3.9	31.8±13.9	–	18
		720	807	41.2±2.2	1.9	13.6± 2.9	–	18
July 15, 1965	11.5	0	485	34.7±0.8	0.9	53.8± 3.2	–	–
		60[a]	420	32.7±1.5	0.9	89.3± 7.6	5	10
		150	541	35.2±1.2	0.8	95.4±18.1	1	13
		240	520	35.2±1.2	0.7	64.1± 9.9	–	13
		480[a]	501	33.7±1.8	0.4	28.0±15.6	–	13
		720	863	40.5±1.7	1.9	20.5± 4.8	–	13
August 12, 1965	12.0	0	1324	48.6±1.4	4.4	64.2± 5.0	–	–
		60[a]	1053	45.8±4.3	1.4	127.3± 5.3	9	15
		90	1353	49.0±2.2	3.8	134.8±10.0	3	21
		480	1150	46.0±2.0	2.9	88.9±20.0	1	24
		720	939	43.4±1.4	1.6	8.8± 1.5	3	33

[a]Five fish used; otherwise 10 were sampled for each recovery period.

Consumption of Food by the Resident Population of Pike, *Esox lucius*, in Lake Windermere[1,2,3]

By L. Johnson[4]

Fisheries Research Board of Canada
Arctic Biological Station, Ste. Anne de Bellevue, Que.

ABSTRACT

The annual consumption of food, the annual production, and the yield of pike in Windermere are determined from equations devised by Beverton and Holt (1957). The determination of the various population parameters is described and these are combined with the results obtained from the experimental determination of food consumption to provide numerical solutions. Prior to the start of heavy exploitation in 1944 the biomass is estimated to have been 8182 kg with an annual consumption of 22,124 kg. After 10 years of fishing the biomass has been reduced to 4349 kg with a consumption of 16,563 kg; this represents a 47% reduction in biomass for a 25% reduction in consumption. These results are discussed in terms of cropping the annual production of the lake.

INTRODUCTION

Windermere, the largest lake in England, has a total area of 14.82 km^2 and a maximum depth of 65 m. It is divided by a chain of islands into north and south basins which have mean depths of 26 and 17.7 m, respectively (Mortimer and Worthington, 1942). Over the past century there has been a gradual eutrophication of the lake, perch (*Perca fluviatilis*) and pike tending to replace char (*Salvelinus alpinus willughbii*) and trout (*Salmo trutta*) as the dominant species (Frost, 1954).

In an endeavour to reduce the pike and perch populations a large-scale experiment was started in 1943. The pike were gillnetted and the perch trapped on an extensive scale; considerable quantities of fish were removed (Worthington, 1949). The experiment was extended in 1950 to include a tagging program, so that, from this date onwards, it is possible to obtain information on the actual numbers in the population. All the pike removed were weighed and measured individually, and from these data, Frost and Kipling (1959) have determined their age and growth.

Pike inhabit much of the inshore water of the lake; netting returns indicate that they are restricted to depths of less than 6.5 m. Frost (1954) made a detailed study of their food at all ages and seasons. She found that at a length

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[2]This work was carried out in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Leeds, England.
[3]This is the second of two parts of a study on food consumption of Lake Windermere pike; the other paper appears in this issue of the Journal.
[4]Present address: Fisheries Research Board of Canada, Freshwater Institute, Winnipeg, Man.

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of 40 mm pike start to supplement their invertebrate diet with small fish; by the time they reach 100 mm invertebrates have lost their significance and fish have become the staple food.

METHODS

The methods employed in this paper are derived from the mathematical models due to Beverton and Holt (1957). The equations they developed for food consumption are based primarily on the growth curve of von Bertalanffy (1934, 1938) and studies of Kostitzin (1939); it is assumed that a population consumes annually the same quantity of food as does one year-class in the whole of its life time. This, of course, is only valid if the year-classes are of constant strength.

The equation for the annual consumption of food (Ξ), by a population of mean annual biomass $\bar{P}w$, is given as:

$$\Xi = \frac{1}{\epsilon_0}\left\{(Q+3KW_{\infty M^{1/3}})\ \bar{P}w^{2/3} - 3K\bar{P}w\right\}\tanh^{-1}\left\{\frac{(Q+3KW_{\infty^{1/3}})\ \bar{P}w^{2/3} - 3K\bar{P}w}{(Q+3KW_{\infty M^{1/3}})\ \bar{P}w^{2/3} - 3K\bar{P}w}\right\}$$

where:

ϵ_0 = The maximum value of the coefficient of utilization of food,

Q = Maintenance energy coefficient (Johnson, 1966),

K = von Bertalanffy constant,

W_∞ = weight at the growth asymptote,

$W_{\infty M}$ = weight at the growth asymptote if food is assimilated at the maximum rate throughout life.

Since the exponent in the maintenance equation (j) is equal to 1, the above equation becomes:

$$\Xi = \frac{1}{\epsilon_0}\left\{\bar{P}w\ (Q-3K) + 3K\bar{P}w^{2/3}\ W_{\infty M^{1/3}}\right\}\tanh^{-1}$$

$$\left\{\frac{(Q-3K)\ \bar{P}w + 3KW_{\infty^{1/3}}\ \bar{P}w^{2/3}}{(Q-3K)\ \bar{P}w + 3KW_{\infty M^{1/3}}\ \bar{P}w^{2/3}}\right\} \quad \ldots\ldots \text{(i)}$$

Before equation (i) can be evaluated it is necessary to compute the biomass ($\bar{P}w$):

$$\bar{P}w = RW_\infty \sum_{n=0}^{3} \Omega_n\ e^{-nK(t_\rho - t_0)}\left\{\frac{1-e^{-(M+nK)\rho}}{M+nK} + \frac{e^{-(M+nK)\rho}\ (1-e^{-(F+M+nK)\lambda})}{F+M+nK}\right\}$$

where:

R = Number of recruits entering the fishing area,

Ωn = the von Bertalanffy summation constant with the following values:

$$\Omega n_0 = 1$$

$$\Omega n_1 = -3$$

$$\Omega n_2 = 3$$

$$\Omega n_3 = -1,$$

M = natural mortality,

F = fishing mortality,

t_ρ = age at recruitment,

$t_{\rho'}$ = age at entry into the exploited phase,

t_0 = arbitrary origin of the growth curve,

t_λ = age at the growth asymptote (in practice the maximum known age),

ρ = $(t_\rho - t_{\rho'})$ duration of preexploited phase,

λ = $(t_\lambda - t_{\rho'})$ fishable lifespan,

n = the exponent in the length–weight equation:

$$W = cL^n,$$

and $\bar{P}w^{2/3}$:

$$\bar{P}w^{2/3} = RW_{\infty^{2/3}} \sum_{n=0}^{3} \Omega'_n \, e^{-nK(t_\rho - t_0)} \left\{ \frac{1 - e^{-(M+nK)\rho}}{M + nK} + \frac{e^{-(M+nK)\rho} \, (1 - e^{-(F+M+nK)\lambda})}{F + M + nK} \right\},$$

with von Bertalanffy summation constant values:

$$\Omega' n_0 = 1$$

$$\Omega' n_1 = -2$$

$$\Omega' n_2 = 1$$

$$\Omega' n_3 = 0$$

and the remainder of the terms as in the preceding paragraph.

Determination of the Parameters

The determination of the biomass depends on a knowledge of the population structure, the mortality (both fishing and natural), the length of the fishable lifespan, and certain constants associated with the von Bertalanffy growth curve. This is a rather lengthy process and only an outline of the procedure is given, the full details being available in the work of Beverton and Holt (1957).

POPULATION

Two years of the Windermere pike fishery were selected for comparison: the first full year of operation, 1944–45, and 10 years later, 1954–55, when the population appears to have stabilised at a new level.

The 1954 population was estimated from the results of the tagging program following the method of Petersen (Ricker, 1958), with an adjustment for the number of tagged fish returned by anglers. The total number of fish vulnerable to the standard gill nets used (five in stretched measure) was estimated to be 858 of which 345 are males and 513 females. Since no tagging records are available for the 1944 season the initial population was estimated by the effectiveness of fishing, assumed to be constant between the 2 years. By this method figures of 1009 males and 1040 females were obtained (95% confidence limits on these population estimates are given in Table I).

Table I. The natural and fishing mortalities, population, and recruitment in 1944 and 1954.

		Mortality			Population		Recruits	
Year		Natural (M)	Nat. and fishing (M & F)	Fishing (F)	No.	95% conf. limits	No.	95% conf. limits
						718		416
	Male	.238	.238	0	1009	1556	584	902
						740		523
1944	Female	.264	.264	0	1040	1604	735	1130
	Total:				2049		1319	
						245		416
	Male	.238	.485	.247	345	532	584	902
1954						365		523
	Female	.264	.687	.451	513	791	735	1130
	Total:				858		1319	

The instantaneous natural mortality of the population was determined from the 1944 catch statistics by plotting the natural logarithm of the population in each age-class. As all fish were not aged at the time of this study, use was made of the table given by Frost and Kipling (1959) for converting length to age. The number of recruits was obtained by adjusting the position of the line of natural mortality to fit the total estimated population and then projecting it backwards to the point where it cuts the y-axis, at zero age. Whilst some error may be incurred in this procedure, since mortality is likely to change with age, it does provide a minimum figure for the number of recruits.

The combined fishing and natural mortality was determined from the 1954 catch statistics in the same way as the natural mortality; then, assuming that the natural mortality has not altered with the passage of time, the fishing mortality can be obtained by subtraction (Table I).

It is further assumed that the number of recruits to the population remained constant between the 2 years under consideration. Justification for this is indicated by the data of Le Cren (1959) who showed that after a rapid rise in the early years of the fishery the number of young pike stabilised at about the level of the 1944 population.

AGE AT CRITICAL STAGES

All the pike in Windermere are confined to the margin of the lake and there is no apparent migration into or out of the area occupied; the age at recruitment (t_ρ) is therefore zero. The age at entry to the fishery is determined

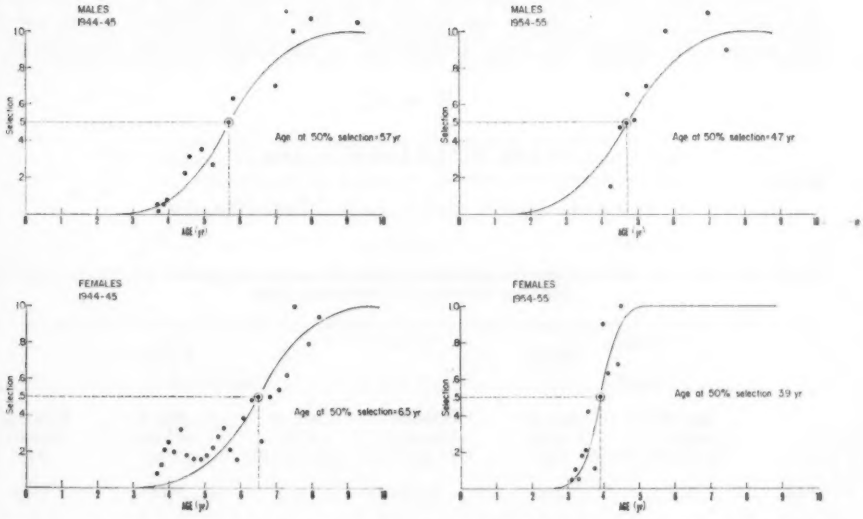

FIG. 1. Ogives describing the mean age of entry into the fishery for male and female pike in the years 1944 and 1954.

by plotting ogives of the fraction of the population in each age-class taken in the gill nets. These fractions were obtained by comparing the actual net catches with the theoretical numbers present of the same age. The age at which 50% of the population becomes vulnerable is taken as the age of entry to the fishery (Fig. 1).

The age of males entering the fishery was reduced from 5.7 to 4.7 years from 1944 to 1954 and the age of females fell 6.5 to 3.9 years over the same period. As these ages have been determined from a length–age relationship it is assumed that the actual growth rates remained constant. If, as seems likely, the growth rate increased somewhat with the heavy fishing, the real ages at entry in 1954 would be slightly less than those recorded.

As the age at entry to the exploited area is zero, the duration of the pre-exploited phase (ρ) is equivalent to the age at entry to the fishery:

$$\rho = t_{\rho'} - t_{\rho}$$

as $t_{\rho} = 0$

$$\rho = t_{\rho'}$$

The age at the growth asymptote (t_{λ}) is theoretically infinite, but the usual practice is to cut it off at the greatest known age of any fish in the population. In this manner ages of 16 years for males and 14 years for females are obtained. The fishable lifespan (λ) is thus the maximum known age minus the age at entry into the fishery ($t_{\lambda} - t_{\rho'}$) (Table II).

GROWTH PARAMETERS

The length at the asymptote of the growth curve (L_{∞}) is given by Frost and Kipling (1959) as 75 cm for males and 100 cm for females from which the asymptotic weight (W_{∞}) was determined by the length–weight relationship:

$$W = cL^{n}$$

$$\text{or Log } W = n \text{ Log } L + \text{Log } c$$

where

$$\text{Log } c = -2.4388 \text{ (males) and } -2.5947 \text{ (females)}$$

TABLE II. The age at entry into the exploited phase, the age at the growth asymptote, and the fishable lifespan of Windermere pike.

	Males			Females		
	Age at entry ($t_{\rho'}$)(=ρ)	Age at infinity (t_{λ})	Fishable lifespan (λ)	Age at entry ($t_{\rho'}$)(=ρ)	Age at infinity (t_{λ})	Fishable lifespan (λ)
1944	5.7	16	10.3	6.5	14	7.5
1954	4.7	16	11.3	3.9	14	10.1

n = 3.1759 (males) and 3.27093 (females)

(data supplied by Kipling, personal communication).

In the subsequent calculations the value of n is taken as 3 in order to facilitate the computation. It is unlikely that any substantial error will be introduced by this procedure.

From the values obtained for the weight at infinity the values of $W_{\infty}^{2/3}$ and $W_{\infty}^{1/3}$ are extracted (Table III).

The maximum possible weight at the growth asymptote ($W_{\infty M}$) which is the parameter describing the growth of a fish assimilating net energy at the maximum possible rate ($1/k$), is given by the equation:

$$W_{\infty M}^{1/3} = \frac{1 - \tanh\,(k\epsilon_0\,\zeta\bar{w}^j)}{3K\bar{w}^{2/3}} + \bar{w}^{1/3}$$

Since it has been shown that for pike the value of the regression coefficient in the maintenance equation is unity, this equation becomes:

$$W_{\infty M}^{1/3} = \frac{1 - \tanh\,(k\epsilon_0\,\zeta\bar{w})}{3K\bar{w}^{2/3}} + \bar{w}^{1/3} \qquad \ldots\ldots \text{(ii)}$$

Substituting in equation (ii) the values obtained for k, ϵ_0, and ζ, the weights of pike at various growth intervals (Johnson, 1966) and the values of K from the following section, estimates of $W_{\infty M}$, $W_{\infty M}^{2/3}$ and $W_{\infty M}^{1/3}$ can be made (Table III).

The von Bertalanffy constant (K) and the theoretical age at birth (t_0), which is the time at which birth would occur if growth were uniform throughout life, were obtained by plotting ($W_{\infty}-w_t$) against w_t, where w_t is the weight

TABLE III. The weight at the growth asymptote under natural conditions and the theoretical maximum weight if food is assimilated at the maximum possible rate throughout life.

	Males	Females
	g	g
W_{∞}	3288.1	8810.0
$W_{\infty}^{2/3}$	221.1	426.42
$W_{\infty}^{1/3}$	14.87	20.65
$W_{\infty M}$	29,389	37,662
$W_{\infty M}^{2/3}$	952.3	1123.6
$W_{\infty M}^{1/3}$	30.86	33.52

at time t (Fig. 2 and 3). Values of K of .22 and .24 were derived for males and females, respectively; similarly the theoretical age at birth was −2.2 years for males and −0.18 years for females.

RESULTS

All the parameters required in the estimation of $\bar{P}w^{2/3}$ being known, this quantity and the biomass could be calculated (Table IV).

FOOD CONSUMPTION

Substituting the above values in equation (i) the basic annual food consumption of the population was obtained (Table V).

However, these consumption figures do not take into account any food consumed for the production of gonads. Le Cren (1962) has suggested that, for perch (*Perca fluviatilis*), the annual elimination of gonad products is about 87% of the annual production of the fish itself. To obtain an estimate of the food consumed for gonad production in pike it has been assumed: in females the egg production is equivalent to the annual production, and in males testes production is half that amount. This gives a figure of 84% for gonad production compared with flesh production, for both sexes combined.

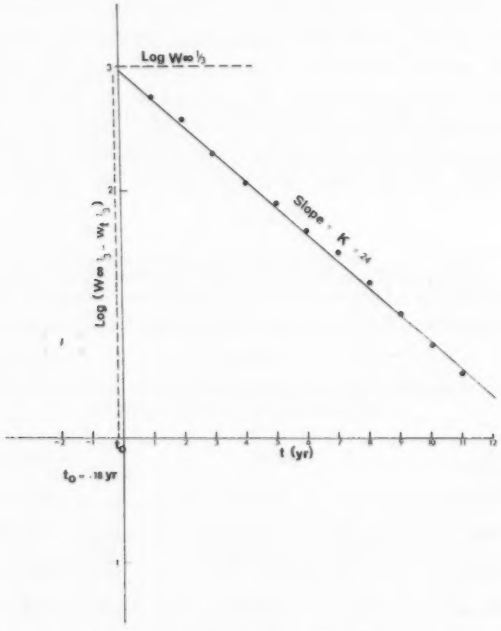

FIG. 2. The determination of the von Bertalanffy constant (K) and the theoretical age at birth (l_0) for males.

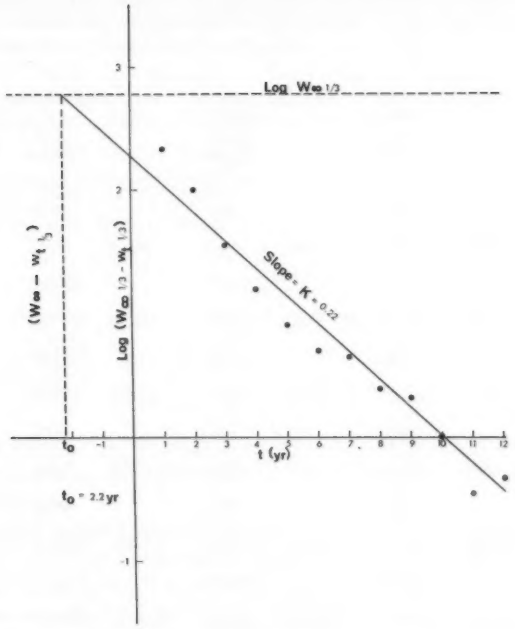

FIG. 3. The determination of the von Bertalanffy constant (K) and the theoretical age at birth (t_o) for females.

TABLE IV. The biomass of Windermere pike and the parameter $\overline{P}w$ calculated for the years 1944 and 1954.

	$\overline{P}w^{2/3}$				Biomass (kg) ($\overline{P}w$)			
	1944	95% conf. limits	1954	95% conf. limits	1944	95% conf. limits	1954	95% conf. limits
		177810		133095		1980		1373
Males	249610	385530	186845	288586	2779	4293	1927	2977
		259643		136974		3844		1724
Females	364891	560989	192497	295947	5402	8306	2422	3724
						5824		3097
Total:					8182	12600	4349	6701

TABLE V. The annual production of Windermere pike (total and in kilograms per hectare), the total annual consumption, and the annual consumption per kilogram of production.

		Basic food (*kg*) 95% conf. limits		Annual prod (*kg*) 95% conf. limits		Total consumption (*kg*) 95% conf. limits		Prod *kg/ha* (420 ha)	Consumption /kg of production
1944			8611		434		4465		
	Males	5575	3971	610	942	6268	9681	1.45	10.28
			8789		1097		11282		
	Females	12352	18990	1542	2371	15856	24377	3.67	10.28
			12760		1531		15747		
	Total:	17927	27601	2152	3313	22124	34058	5.12	10.2
1954			2965		396		3415		
	Males	4162	6429	557	860	4794	7406	1.33	8.61
			6559		799		8374		
	Females	9218	14171	1123	1726	11769	18093	2.67	10.47
			9524		1195		11789		
	Total:	13380	20600	1680	2586	16563	25499	4.00	9.86

The annual production can be determined from the equation:

$$A.P. = 3K\,(W_{\infty^{1/3}}\,\bar{P}w^{2/3} - \bar{P}w).$$

The food equivalent to this production was obtained by dividing by the efficiency of utilization (ϵ) and the total consumption was derived by adding the specified amount to the basic consumption (Table V).

In order to determine the rate of cropping within the lake these results may be apportioned: (1) on the basis of kilograms of food consumed per hectare over the whole lake surface (1482 ha); (2) on the basis of the area occupied by the pike (420 ha); (3) the food consumed per kilogram of biomass (Table VI); and (4) the food consumed per kilogram of production (Table V). In order to keep these tables simple the 95% confidence limits have been omitted, but they may be readily obtained by the appropriate division.

During the course of the pike fishery both the biomass and the food consumption diminished: the biomass from 8182 to 4349 kg, whilst the food consumption fell from 22,124 to 16,563 kg. Thus, a reduction of 50% in the biomass resulted in a mere 25% drop in food consumption. The reason for this big difference would appear to be the large increase in production per unit biomass: from .26 kg in 1944 to .39 in 1954 — a 47% increase. This

TABLE VI. The consumption of food in kilograms per hectare and the consumption per kilogram of biomass.

Year		Total food (*kg*)	Biomass/ ha (420 *ha*)	Consumption/ ha (1482 *ha*)	Consumption/ ha (420 *ha*)	Consumption/ kg biomass
	Males	6268	6.61	4.23	14.92	2.25
1944	Females	15856	12.86	10.70	37.75	2.93
	Total:	22124	19.47	14.93	52.67	2.70
	Males	4794	4.59	3.23	11.41	2.49
1954	Females	11769	5.77	7.94	28.02	4.86
	Total:	16563	10.36	11.17	39.44	3.81

increase in the rate of production and the relatively small decrease in food consumption emphasize the difficulty in reducing predation by the removal of the adult predator.

The dissimilar growth pattern of males and females results in the males forming 34% of the total biomass in 1944. In 1954, males formed 44% of the biomass, this increase being due to the fishery having less effect on the small, slow-growing males, than on the larger females.

The rate of cropping fell between the 2 years from 14.93 to 11.17 kg/ha when the whole surface area of the lake is considered; when only the area occupied by the pike is taken into account the cropping was reduced from 52.67 to 39.44 kg/ha. Mortimer (1954) estimates that the mean level of production in English waters is approximately 23.6 kg/ha. It seems probable that Windermere has a production of this order of magnitude. The production of perch is about 15.5 kg/ha (Le Cren, 1962) and other species contribute a very much smaller proportion. Consumption by the pike thus seems to be greatly in excess of the production within the area which they inhabit. The whole of the lake is apparently helping to support the pike population without the pike having to forage beyond the shallows. This is well illustrated by Frost's work of feeding habits: she showed that during the winter months, when some char enter the shallower waters to spawn, they are heavily preyed on by the pike, whilst at other times of the year they escape capture entirely. Similarly, during the summer, perch enter shallow water and are consumed in quantity, but are less important in winter when they live in the deeper parts of the lake.

In the period prior to the introduction of the netting program, the pike appear to have consumed some 63% of the total production of the lake and this was reduced to 47% in later years. It may be noted that in 1944 the upper confidence limit for consumption (22.98 kg/ha) is almost equal to the estimated production. Thus, in spite of the minimal figures used in this study, it seems

that pike consumes a high percentage of the total production, although itself forming only a small fraction of the total biomass.

The consumption of food per kilogram of biomass increased appreciably between 1944 and 1954: from 2.70 kg to 3.81 kg. The increase is again linked with the increase in production per unit biomass. It is now possible to hazard an answer to the old question: how much does a fish eat? It would seem that a mean pike will consume annually between three and four times its mean annual weight depending upon the intensity of exploitation.

The consumption per kilogram of production also has interesting aspects. In 1944 consumption amounted to 10.28 kg per unit production falling to 9.86 kg in 1954. This may be compared with the "gross conversion" figure of 3.42 obtained for the small experimental pike (Johnson, 1966). The difficulties inherent in using such a coefficient for comparison between species is well illustrated. In such a figure it is the total amount of food used for maintenance which dominates. In the small pike 30% of the total intake is used for maintenance whereas in the whole population it rises to 78%.

The advantages of large size to a predator are thus, to a degree, offset by its large maintenance requirement, and the relatively small fraction of the total food which is available for growth. In these circumstances it is to its advantage to minimize the maintenance requirement and maximize the efficiency of conversion. That this is exactly what is occurring is shown by the results of the feeding trials.

As a final check to the general accuracy of the figures presented in this paper it is possible to estimate the yield of the fishery (Yw) from the equation:

$$Y_w = RFe^{-M\rho} W_\infty \sum_{n=0}^{3} \frac{\Omega_n e^{-nK(t\rho'-t_0)}}{F + M + nK} (1 - e^{-(F+M+nK)\lambda}).$$

Substituting in this equation the data for 1954, a yield of 238 kg of male fish and 468 kg of females was obtained (Table VII). This may be compared with an actual yield of 219 kg of males and 545 kg of females.

TABLE VII. The calculated yield of pike from the 1954 Lake Windermere fishery and the actual catch in that year.

	Fishable biomass ($\bar{P}'w$)	Fishing mortality (F)	Yield (Yw)	Actual catch
Males	888.75	.247	219.0	237.6
Females	793.80	.687	545.3	468.3
Total:	1682.55		764.3	705.9

It may be contended that the closeness of this result is due to circularity in the argument. This is true insofar as the calculations have led back to the starting point of the catch statistics, but considerable information has been generated in the process, with apparently no great accumulation of error.

It cannot be claimed that the figures presented above are correct to the nearest kilogram as various approximations and assumptions have been made, but it may be claimed that the results seem to represent a reasonable approximation to the field situation. Even more important, perhaps, is the insight provided into the mechanisms of the feeding pike and the effect of heavy fishing on total consumption.

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Developmental Rate and Vertebral Number in Salmonids[1,2]

By E. T. Garside[3]

Department of Biology, Dalhousie University, Halifax, N.S.

ABSTRACT

Eggs of the brook trout and the rainbow trout were incubated at various constant levels of temperature and dissolved oxygen to test the hypothesis that vertebral number in fish is controlled, within limits, by the developmental rate. The mean vertebral number in both species decreased with increased rate of development which was caused by higher temperature and conversely the mean vertebral number in both species increased with decreased rate of development. The number of vertebrae is shown to be inversely related to the rate of development, expressed as the reciprocal of the number of days in the period of numerical determination of vertebrae in each species. The slopes of the regressions are −0.45 and −1.15 for brook trout and rainbow trout, respectively, and are perhaps, characteristic of the species. Variations in the usual type of trend of vertebral number with different developmental conditions, as reported by some investigators, are discussed and a possible explanation for some of these variations is proposed.

INTRODUCTION

The first experimental studies on the influence of environmental variables, especially temperature, on the numbers of certain meristic structures in fishes were conducted by Schmidt (1919) who was able to demonstrate significant differences in the vertebral number among lots of brown trout (*Salmo trutta* L.) which had been maintained at different temperatures during the period of embryonic development. He concluded from his studies that while the vertebral number was controlled directly by genetic mechanisms there was some modification in the numbers resulting from selections of temperature-specific genotypes.

Hubbs (1926) presented a new approach to this phenomenon, stating that the vertebral number in fishes was within genetically determined limits, a function of the rate of development rather than the result of environmentally controlled selections of certain genotypes which are characterized by definite numbers. However, Hubbs did not test this hypothesis experimentally.

In the present study various lots of salmonid eggs were incubated at various levels of constant temperature and dissolved oxygen in order to produce a broad range of developmental rates and vertebral numbers. The propo-

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[2]Contribution from the Laboratory for Experimental Limnology, Maple, Ontario, which is operated jointly by the Department of Zoology, University of Toronto, and the Research Branch, Ontario Department of Lands and Forests. This paper is extracted from a portion of a thesis submitted to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1960.

[3]Present address: Department of Biology, Dalhousie University, Halifax, N.S.

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sition of Hubbs that vertebral number is a function of the developmental rate, especially of the rate during the period of vertebral formation, was subsequently tested.

Consideration has also been given to results obtained by some investigators which contravene the findings of others, including those of the present study, that the number of vertebrae decreases with increased temperature. In some instances the lowest number is found at an intermediate temperature, and in a few cases the number of vertebrae has shown only an increase with increasing temperature. An explanation is presented to at least partially account for these differences.

MATERIALS AND METHODS

Eggs and Apparatus

A description of the procedure including sources and quantities of eggs, dates of operation, and control of experimental environments for these experiments has been recorded by Garside (1966) in a report of another aspect of this study.

In the autumn of 1957, 16 lots of approximately 750 eggs of the brook trout (*Salvelinus fontinalis* Mitchill) were incubated in 16 different experimental environments which were the combinations of four temperatures, 2.5, 5.0, 7.5, and 10 C, and four levels of dissolved oxygen approximating 2.5, 3.5, 4.5 ppm, and a control level approaching the values for air-saturation at each temperature.

A similar experiment was performed on 11 lots of about 1200 eggs of rainbow trout (*Salmo gairdneri* Richardson), in the spring of 1958. Four temperatures, 2.5, 5.0, 7.5, and 10 C were also used in this experiment but only two reduced levels of dissolved oxygen were used; they approximated 2.5 and 3.5 ppm. At 2.5 C only one reduced level approximating 2.5 ppm was employed.

Treatment of the embryos with water of reduced oxygen content was terminated, in each instance, when the embryos were in a late stage of development, at the beginning of notochordal flexure. These details are summarized in Table I for brook trout and in Table II for rainbow trout. However, incubation temperatures were maintained until hatching was completed in each lot. The lots of alevins were maintained thereafter in separate 70 l. aquaria supplied with running tap water at approximately 9 C.

Developmental Studies

The progress of development in both brook trout and rainbow trout was followed by recording the times required by embryos in samples taken from the various lots to attain each of the 18 embryonic stages in the series outlined by Garside (1959). Samples of eggs were taken at daily intervals for future examination and preserved in a solution of one part each of formalin, glycerine, and glacial acetic acid, three parts ethanol and four parts distilled water.

TABLE I. Experimental environments of the lots of brook trout in the 1957 experiment together with mean vertebral counts of the lots and the numbers of individuals with abnormal vertebrae. The four lots of embryos incubated at 5 C and the lot of embryos incubated at 4.2 ppm of dissolved oxygen at 10 C were lost accidentally prior to the counts of vertebrae.

Mean temp *C*	Mean dissolved O_2, *ppm*	Oxygen treatment in days	Number in lot	Mean vertebral number	Number with fused vertebrae	Number with complex vertebrae	Total with abnormal vertebrae
2.5±.1	2.6±.2	77	71	58.35±0.14	2	0	2
2.5±.1	3.7±.2	77	9	57.78±0.25	0	0	0
2.5±.1	4.5±.2	77	20	57.50±0.23	0	0	0
2.5±.1	10.5±.1[a]	–	32	57.13±0.11	3	0	3
7.5±.1	2.6±.2	53	36	56.50±0.15	0	0	0
7.5±.1	3.4±.2	53	50	56.58±0.12	0	0	0
7.5±.1	4.3±.1	53	18	56.27±0.32	0	0	0
7.5±.1	10.3±.2[a]	–	50	56.02±0.10	1	0	1
10.0±.1	2.5±.1	45	50	55.66±0.20	7	1	8
10.0±.1	3.3±.2	45	50	55.44±0.21	13	0	13
10.0±.1	4.2±.1	45					
10.0±.2	10.2±.2[a]	–	50	55.14±0.17	2	1	3

[a]Control level of dissolved oxygen.

TABLE II. Experimental environments of the lots of rainbow trout embryos in the 1958 experiment together with mean vertebral counts and numbers of individuals with abnormal vertebrae.

Mean temp *C*	Mean dissolved O_2, *ppm*	Oxygen treatment in days	Number in lot	Mean vertebral number	Number with fused vertebrae	Number with complex vertebrae	Total with abnormal vertebrae
2.5±.1	2.9±.3	80	16	64.75±0.25	3	2	5
2.5±.1	11.3±.1[a]	–	48	64.21±0.17	1	6	7
5.0±.1	2.8±.2	65	76	63.88±0.13	2	0	2
5.0±.1	3.9±.1	65	95	64.06±0.16	0	0	0
5.0±.1	11.3±.1[a]	–	15	63.93±0.26	0	0	0
7.5±.1	2.6±.2	45	44	63.75±0.20	0	3	3
7.5±.1	3.7±.2	45	12	63.67±0.28	0	0	0
7.5±.1	10.6±.2	–	96	62.64±0.13	0	2	2
10.0±.1	2.5±.2	34	15	63.27±0.17	0	6	6
10.0±.1	3.5±.2	34	63	63.19±0.12	11	2	3
10.0±.1	10.5±.1[a]	–	59	61.83±0.16	1	10	11

[a]Control level of dissolved oxygen.

VERTEBRAL COUNTS

Arithmetic means of vertebral counts were determined for the lots of brook trout and rainbow trout. The "method of standard errors" as applied to the graphic presentation of means (Hubbs and Hubbs, 1953) was used

as the primary test for ascertaining the statistical significance of differences among the various means. The "t–test" was used in cases in which decisions could not be reached by the graphic method.

BROOK TROUT

The vertebral counts of brook trout were taken radiographically. The smallest fish were about 6 cm long when they were killed, marked with numbered tags, and preserved in 10% formalin. Radiographs were made on Ilford "Ilfex" plates used on a standard diagnostic machine. A satisfactory exposure was 0.5 sec at a distance of 75 cm using 120 kv and 25 amp. The processed plates were examined on an illuminated glass-top tracing table. A hand lens was used to facilitate counting. All vertebrae between the skull and the urostyle were counted.

Two types of abnormal vertebrae which occur in the vertebral column of fish were identified. One type is the serial fusion of two or more centra. These elements are generally readily distinguished and each usually has a neural arch and a haemal arch. Each such element was counted as a single vertebra. When it was not possible to determine the number of centra in a synostosis the count was not recorded. The other type of abnormality is the "complex vertebra" (Kändler, 1932, 1935). In this type of vertebra the neural arch, the haemal arch or both have been duplicated on a single centrum. A complex vertebra was counted as a single vertebra in this study.

RAINBOW TROUT

Only rainbow trout larger than 8 cm were prepared radiographically for vertebral counts. Shorter fish were stained *in toto* using the potassium hydroxide and Alizarin technique described by Hollister (1934). The vertebrae were readily counted using a stereoscopic microscope. It was necessary to remove the left operculum to see the first few vertebrae.

RESULTS

Brook Trout

Mean vertebral counts for the lots of brook trout are given in Table I. Failures in the water supply to the aquaria containing all of the lots of brook-trout alevins which had been incubated at 5 C and also to the aquarium containing those incubated at 7.5 C and 4.2 ppm of dissolved oxygen caused total mortality before vertebral counts were made. In the remaining lots the highest vertebral numbers occurred at 2.5 C, the lowest temperature. The lowest numbers of vertebrae occurred in the lots at 10 C, the highest temperature. The differences among the mean counts of the control lots at the three temperatures are significant in every instance ($P_{0.01}$). Within each of the levels of temperature there were differences in vertebral numbers which were correlated with the level of dissolved oxygen. The highest counts were obtained from the lots which were incubated at the lowest level of oxygen. At 2.5 C the difference between the means of the lots which were incubated

at 2.6 ppm of oxygen and 10.5 ppm of oxygen is also significant ($P_{0.01}$). The means at the low and high levels of oxygen at 7.5 C are also significantly different ($P_{0.05}$). The difference between these means at 10 C is scarcely significant ($t = 1.95$; $P_{0.05} = 1.98$).

The incidence of brook trout with serial fusions and/or complex vertebrae in their vertebral columns is greater at the terminal temperatures of incubation than at the intermediate level of 7.5 C.

Rainbow Trout

Mean vertebral counts of the lots which were incubated in the 1958 experiment are shown in Table II. The total counts show the same trend as was found in the brook trout incubated at the same temperatures, the highest mean count occurring at 2.5 C and the lowest mean count occurring at 10 C. Within each level of temperature there are differences in mean counts which, in the lots at 2.5, 7.5, and 10 C, are correlated with differences in the levels of oxygen. At 2.5 C the decrease of 0.54 vertebra from the lot at 2.9 ppm to the lot at 11.3 ppm of oxygen is not significant ($P_{0.2}$), but the former was calculated from only 16 fish which, perhaps, is not a reliable sample. The lots at 5 C do not conform with the pattern observed in the groups at the other temperatures. Also, the 5 C lots do not have significant differences among their mean counts, ($P_{0.4}$). At 7.5 C the difference between mean counts at low and high levels of oxygen is 1.11 vertebrae which is significant ($P_{0.02}$). There is significant difference ($P_{0.01}$) of 1.44 vertebrae between the lots of rainbow trout incubated at low and high levels of oxygen at 10 C.

Both serial fusions and complex vertebrae were more prevalent at the low and high temperatures than at the intermediate temperatures of incubation.

DISCUSSION

The investigations of Schmidt and of Hubbs in the years immediately preceding and following 1920 stimulated further research on variations in vertebral numbers within species of fishes. A list of regulated experiments, in which there were observed changes in vertebral number when embryos were incubated at various levels of temperature and other factors, is given in Table III. The pertinent literature contains many other reports of variations in vertebral number within species taken from their natural environments. Some of these are listed in Table IV. However, these environmental conditions were often quite variable and in most instances the levels or intensities of the essential factors such as temperature and dissolved oxygen were not especially well recorded. Thus, in these latter cases it is rather difficult to form correlations between gradients in the levels of environmental factors and variations in vertebral numbers. Therefore, rather less consideration will be given to such reports in this discussion.

Gabriel (1944) working with the killifish (*Fundulus heteroclitus*) was the first investigator to correlate vertebral number with a measure of the

TABLE III. A list of laboratory experiments conducted by several investigators to study the influence of various environmental factors on the number of vertebrae in various species of fish.

Author	Species	Environmental factor	Vertebral alteration
Schmidt (1919)	*Salmo trutta* (brown trout)	Temperature	Lowest number at intermediate temperature; number increased at lower and higher temperatures.
Schmidt (1920)	*Zoarces viviparus* (blenny)	Temperature or salinity	Number decreased with increased temperature or salinity.
Mottley (1937)	*Salmo* spp. (trout)	Temperature or oxygen	Number decreased with increased temperature (possibly lower oxygen level).
Gabriel (1944)	*Fundulus heteroclitus* (top-minnow)	Temperature	Number decreased with increased temperature.
Tåning (1944, 1950, 1952)	*Salmo trutta* (sea trout)	Temperature	Lowest number at intermediate temperature (however, not consistently).
		Oxygen	Increased number with decreased level of oxygen.
		Carbon dioxide	Decreased number with increased level of carbon dioxide.
Hempel (1953)	*Clupea harengus* (herring)	Temperature	Number decreased with increased temperature.
Marckmann (1954)	*Salmo trutta* (sea trout)	Temperature	Lowest number at intermediate temperatures.
McHugh (1954)	*Leuresthes tenuis* (grunion)	Light intensity	Lowest number at highest intensity.
Lindsey (1954, 1958)	*Macropodus opercularis* (paradise fish)	Temperature	Lowest number at intermediate temperature.
	Oncorhynchus nerka (kokanee)	Light duration	Lowest number at longest duration.
Orska (1956)	*Salmo trutta* (sea trout)	Temperature	Number decreased with increased temperature.
	Salmo gairdneri (rainbow trout)	Temperature	Number decreased with increased temperature.
Seymour (1956)	*Oncorhynchus tshawytscha* (chinook salmon)	Temperature	Lowest number at intermediate temperature (however, not consistently).
Blaxter (1957)	*Clupea harengus* (herring)	Temperature	Lowest number (myomeres) at highest temperature.
Molander and Molander-Swedmark (1957)	*Pleuronectes platessa* (plaice)	Temperature	Lowest number at intermediate temperature.
Smirnov (1954)	*Oncorhynchus nerka* (kokanee)	Temperature	Number of somites formed prior to closure of bastopore was lower at higher temperatures.
Canagaratnam (MS, 1959)	*Oncorhynchus* spp. (salmon)	Light intensity	Results variable among various species.
	Salmo gairdneri (rainbow trout)	Light duration	Results variable among species.
	Oncorhynchus nerka (kokanee)	Temperature	Number increased with increased temperature (8 and 12 C).

TABLE IV. A list of the results of some field studies of variations in vertebral numbers[a] in fishes correlated with certain conditions of the environment, especially temperature.

Author	Species	Environmental condition	Vertebral alteration
Schmidt (1917, 1920, and 1923)	*Zoarces viviparus* (blenny)	Temperature/ salinity	Number decreased with increased temperature, decreased salinity.
Hubbs (1922, 1925)	*Notropis* spp. (shiner)	Temperature	Number (caudal vertebrae) decreased with increased temperature.
	Clupea pallasii (Pacific herring)	Temperature	Number decreased with increased temperature.
	Sardinia caerulea (sardine)	Temperature	Number decreased with increased temperature.
	Engraulis mordax (anchovy)	Temperature/ salinity	Number decreased with increased temperature, decreased salinity.
Dannevig (1932, 1933)	*Gadus morhua* (cod)	Temperature/ light intensity	Number decreased with increased temperature and increased intensity.
Runnstrøm (1933)	*Clupea harengus* (herring)	Temperature	Number decreased with increased temperature.
De Buen (1934)	*Sardinia pilchardus* (sardine)	Temperature	Number decreased with increased temperature.
Lissner (1934)	*Clupea harengus* (herring)	Temperature	Number decreased with increased temperature.
Tester (1938)	*Clupea pallasii* (Pacific herring)	Temperature	Number decreased with increased temperature.
Heuts (1947)	*Gasterosteus aculeatus* (stickleback)	Salinity	Number decreased with decreased salinity.
McHugh (1954)	*Clupea pallasii* (Pacific herring)	Temperature	Number decreased with increased temperature.
Day (1957)	*Clupea harengus* (herring)	Temperature	Number decreased with increased temperature.
Vernon (1957)	*Oncorhynchus nerka* (kokanee)	Temperature	Number decreased with increased temperature.

[a]There are many other records of vertebral numbers taken from natural populations but nearly all are without measurement of even a single variable of the environment. These reports relate vertebral counts only to geographic location. An extensive list of such reports is given by Molander and Molander-Swedmark (1957).

time of development which he took as the time from fertilization to the absorption of the yolk. Mean vertebral counts were highest at 13.5 C, lowest at 24.5 C, and intermediate at 18.5 C, while the developmental rates, as indicated by developmental times, were inversely related to these temperatures. Studies of the vertebral counts of the individuals within any of these levels of temperature revealed that there was also a gradual increase in vertebral number with increased time of development among those individuals. He concluded that the number of vertebrae was related to the rate of development, subject to the degree of "intrinsic lability" of the individual.

While other investigators since Gabriel have associated differences in vertebral numbers with different developmental temperatures in several species of fish, they have not taken measurements of developmental time into consideration in most instances. Tåning (1946, 1952) attempted to define the period during which the vertebral number was plastic. However, he measured the progress of development in day-degrees or thermal units. This is not a valid procedure since the rate of embryonic development in fish is not a linear function of temperature over a wide range (Kinne and Kinne, 1962; Garside, 1966) and so the thermal unit or day-degree does not indicate a constant amount of development through the biokinetic range of temperatures.

Another approach was that of Marckmann (1954) which took into account the time required for development also by assuming that developmental metabolism could be expressed by day-degrees. After selecting an arbitrary developmental zero of –0.5 C he plotted the numbers of day-degrees for the development of embryos of sea trout (*Salmo trutta* L.) at 3, 6, 9, and 12 C. The least number of day-degrees was obtained at 6 C as was the lowest mean number of vertebrae. He suggested that the vertebral number was governed by the rate of developmental metabolism of the embryo and that the embryos at 6 C had the "most economical metabolism" and so, the fewest vertebrae. Barlow (1961) has shown that Marckmann's thesis was improperly derived. He plotted Marckmann's data using the Kelvin scale for obtaining the products and has shown, instead, that the developmental rate actually increased steadily from the lowest to the highest temperature. Thus, the similarity in form of Marckmann's curves for day-degrees and for vertebral numbers, at various temperatures, was dependent upon the selection of –0.5 C as the developmental zero.

Other environmental components which are capable of modifying the developmental rate have also been shown to influence the number of vertebrae in the embryos of fish. Hypoxia retards developmental rates in embryos of fish (Kinne and Kinne, 1962; Garside, 1966) and has also been associated with changes in vertebral number by Tåning (1952), Seymour (MS, 1956), and by the results of the present study. Light has also been shown to modify the developmental rate and to influence the number of vertebrae. Canagaratnam (MS, 1959) has shown that development was accelerated by longer durations of light and higher intensities of light and that lower mean vertebral counts occurred under these conditions in salmon, *Oncorhynchus* spp.

The foregoing information leads to the generalization that levels of constituent factors of the environment which have a retarding effect on the rate of development, thereby protracting the period of development, are associated with increased numbers of vertebrae while those levels of factors which accelerate development are associated with reduced numbers of vertebrae as stated by Hubbs.

Certain minor refinements can be made to this generalization with data from the present study. The first step is to determine the period of time which constitutes the period of differentiation for vertebrae. This period should

be more pertinent to the relationship of developmental rate and vertebral number than is the entire period of embryonic development.

Following the studies of Price (1934a) on the lake whitefish (*Coregonus clupeaformis* Mitchill) and of Battle (1944a) on the Atlantic salmon (*Salmo salar* L.) and observations of embryos collected in the present study, Stage 4 or one-quarter epiboly has been chosen as the stage at which metameric segmentation of the mesoderm begins. The assumption is that this is the earliest stage at which presumptive vertebral tissue could be affected by environmental components, such as temperature. Tåning (1946, 1952) states that according to his temperature-transfer experiments the period of determination of vertebrae begins well in advance of axiation (pregastrulation). However, his measurements of stages of development are based upon the numbers of thermal units which have accumulated and this is an improper approach when more than one temperature is considered. Also, there are inconsistencies in his results which make his conclusions questionable.

The stage at which the determination of the vertebral number is completed has not yet been determined with even slight precision. Price (1934b) and Battle (1944a) have stated that the formation of somites is completed shortly before one-half of the total period of incubation, in the lake whitefish and the Atlantic salmon, respectively. Tåning (1946) found that the number of vertebrae in sea trout could be altered by temperature-transfers to a stage which, according to his illustrations, corresponds to Stage 14 for the closely related species used in the present study. This is somewhat later in the developmental period than the stage indicated by Price and by Battle for somites, also in closely allied species. However, Tåning has again used the accumulation of equal numbers of thermal units as the criterion for making the transfers between temperatures. There is a lag between the differentiation of somites and the formation of vertebrae such as that given by McBride (1932) and Nelsen (1953). To make some allowance for this lag, Stage 16, notochordal flexure, has been selected as the stage of development by which the determination of vertebral number is completed. At this stage the notochord, around which the sclerotomic primordia of the vertebrae condense, terminates posteriorly and flexes dorsally into its final shape. The hypural elements and the rays of the caudal fin begin to appear shortly thereafter, and it would be reasonable to assume that the vertebrae upon which these structures articulate would have been established.

The mean vertebral counts for the brook trout in the 1957 experiment and for the rainbow trout in the 1958 experiment can be plotted with their respective developmental rates which are the reciprocals of the differences in time between Stage 4 and Stage 16, which were recorded by Garside (1966). These are the stages chosen as the beginning and the end, respectively, of the period of vertebral determination. The calculated rates are given in Tables V and VI. The relationships for the brook trout and the rainbow trout in these experiments are shown in Fig. 1. There is a definite trend indicating that an increase in the developmental rate is accompanied by a decrease in vertebral

TABLE V. The relation of the rate of development of embryos of the brook trout to the number of vertebrae. The rate of development during the period of determination of the vertebrae is given as the reciprocal of the number of days required by each lot of embryos to pass from Stage 4 to Stage 16. Differences obtained from data of Garside (1966).

Mean temp C	Mean dissolved O_2, *ppm*	Period of determination	Reciprocal × 100	Vertebral number
2.5	2.6	63	1.58	58.58
	3.7	59	1.70	57.78
	4.5	56	1.79	57.50
	10.5	50	2.00	57.13
7.5	2.6	32	3.11	56.50
	3.4	30	3.34	56.58
	4.3	27	3.71	56.27
	10.3	24	4.17	56.02
10.0	2.5	26	3.86	55.66
	3.3	22	4.55	55.44
	4.2			
	10.2	19	5.27	55.14

TABLE VI. The relation of the rate of development of embryos of rainbow trout to the number of vertebrae in the 1958 experiment. The rate of development during the period of determination of vertebrae is given as the reciprocal of the number of days required by each lot to pass from Stage 4 to Stage 16. Differences obtained from data of Garside (1966).

Mean temp C	Mean dissolved O_2, *ppm*	Period of determination	Reciprocal × 100	Vertebral number
2.5	2.9	53	1.89	64.75
	11.3	50	2.00	64.21
5.0	2.8	36	2.78	63.88
	3.9	35	2.86	64.06
	11.3	29	3.45	63.93
7.5	2.6	26	3.85	63.75
	3.7	25	4.00	63.67
	10.6	18	5.55	62.64
10.0	2.5	24	4.17	63.27
	3.5	18	5.55	63.19
	10.5	13	7.69	61.83

number in both species. This relationship holds regardless of whether the developmental rate is controlled by temperature or limited by a reduced level of dissolved oxygen. The slopes for the regression lines are −1.15 for brook trout and −0.45 for rainbow trout. Perhaps these values are characteristic of the species. It is of interest to note in passing that while the overall period of embryonic development to hatching in rainbow trout is more rapid than that of brook trout, the period of vertebral determination is relatively greater

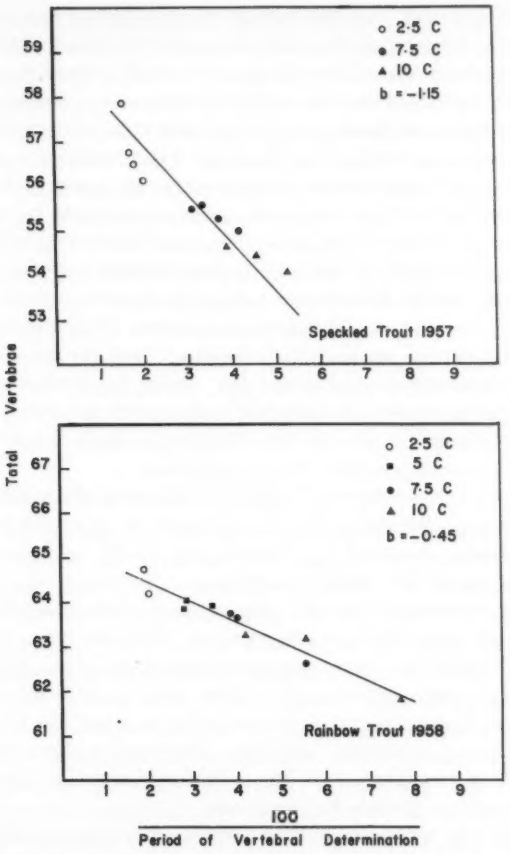

FIG. 1. Mean numbers of vertebrae in the various lots of brook trout and of rainbow trout, incubated in the 1957 and 1958 experiments, plotted with their respective rates of development, expressed as the reciprocals of the times for the periods of numerical determination of vertebrae. This period of determination has been defined in the text as the time, in days, from Stage 4 to Stage 16 in the series of embryological stages, described by Garside (1959) and in the present text.

and also that the number of vertebrae is greater in the rainbow trout. However, this observation does not warrant further consideration at this time on the basis of only one experiment on only two species.

VARIATION IN TRENDS OF VERTEBRAL NUMBERS

Among the reports listed in Table III there are three types of trend in numbers of vertebrae including the trend reported in this study, namely

the decline in vertebral number which accompanies accelerated developmental rate. This is the trend most commonly observed. The reverse trend has also been reported in certain instances. In still others the trend involves a decrease in the vertebral number with accelerated development to an intermediate level, and then as development continues to accelerate beyond this level the number of vertebrae begins to increase. Thus, when the mean vertebral numbers are plotted with their respective developmental temperatures, a V-shaped curve obtains. Such curves have been reported by several investigators as shown in Table V for several species. Such V-shaped trends have not been reported in clines in nature. Lindsey (1954) has suggested that two mechanisms might be involved, one being dominant at lower temperatures while the other is dominant at higher temperatures. If such unspecified mechanisms do exist they are apparently regulated only by temperature. Other factors such as dissolved oxygen and light, which have been shown to affect the developmental rate and the vertebral number do so in only one direction, that is, retarded development (within limits) produces more vertebrae and accelerated development produces fewer vertebrae.

Barlow (1961) has proposed a model to illustrate the possible interaction between growth and differentiation in embryos of fish which is in keeping with the hypothesis advanced by Hubbs and which is consistent with the V-shaped curves found by some investigators. In this model, differentiation of vertebrae has a low coefficient of temperature while general growth of the embryo has a high coefficient of temperature. Growth, then, tends to be retarded relatively more at lower temperatures than does differentiation of vertebrae and so more vertebrae will form over a relatively longer period of time. In cases of V-shaped curves for vertebral number, the apex of the curve is interpreted as being coincident with the inflection of the coefficient of temperature. This model requires that the points of inflection for the two coefficients of temperature coincide rather closely.

There exists the possibility that increases in vertebral number with increasing temperature including those increases in vertebral number that form the ascending or right branch of a V-shaped curve result in part from the method of counting vertebrae. Various investigators have established different criteria for defining abnormal vertebrae and for their enumeration, particularly in the case of the complex vertebra. This disagreement arises from the failure of these workers to adopt the same criteria for defining a vertebra. According to McBride (1932) and Nelsen (1953) the first step in the differentiation of vertebrae is the serially discontinuous investment of the notochord by tissue of the sclerotome which forms the centra. This means that the centrum is the basic unit. In spite of this, some investigators resort to the use of the number of neural and/or haemal arches as the vertebral count on the assumption that these are better indicators of segmentation. Schmidt (1919) found that by counting a complex vertebra as one and one-half vertebrae the individuals with a complex vertebra then had the same counts as the mean count for their normal siblings. Tåning (1944) counted a

complex vertebra as two vertebrae if the haemal arch was duplicated. Molander and Molander-Swedmark (1957) counted a complex vertebra as two if either arch was duplicated on a single centrum. Examination of their data for plaice (*Pleuronectes platessa* L.) reveals that the increases in vertebral number at both low and high temperatures are accompanied by considerable increases in the percentage frequency (36–50%) of individuals with complex vertebrae which they have chosen to count as two vertebrae. Thus, they have considerably increased the mean counts for vertebrae at the low and high temperatures thereby accentuating if not, in fact, creating the V-shape of the curves. Schmidt (1919) and Tåning (1944), although they have omitted their data, in each case, can be presumed to have included additional vertebrae in their counts since both considered a complex vertebra as being more than one vertebra. In the present study there was some increase in the frequency of abnormal vertebrae at higher developmental temperatures as shown in Tables I and II but the vertebrae were enumerated according to the number of discernible centra, thus, a complex vertebra was counted as a single unit.

Selective Mortality and Vertebral Number

As indicated previously Schmidt (1919) first considered that variation in vertebral number in fish resulted from selection of certain genotypes which produced fixed numbers of vertebrae. The possible role of selection by environmental factors such as temperature in the determination of vertebral number is indeed most difficult to evaluate. Fish die in the embryonic phase from a variety of causes such as mechanical agitation (Battle, 1944b; Smirnov, 1954), diseases, especially infection by fungi, and at hatching during which time there is a rapid and large increase in the oxygen requirements of embryos (Lindroth, 1942; Hayes et al., 1951; Garside, 1959) which is often not satisfied especially at higher temperatures. Mortality is regularly high in lots of embryos reared in laboratory conditions (Seymour, 1950; Molander and Molander-Swedmark, 1957; Canagaratnam, MS, 1959) and can usually be ascribed to one or more of the above conditions. The matter of the selection of specific genotypes by various levels of environmental components has been considered by Lindsey (1962) and it is his opinion also that variations in the numbers of serial structures are not brought about primarily by differential mortality. While the possibility of selective mortality cannot be completely precluded no hypotheses have been advanced in the literature to relate the death of an embryo to the acquisition of a particular number of vertebrae.

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Some Methods for Estimating Exploited Populations[1]

By K. Radway Allen

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

ABSTRACT

Three methods of estimating population and one of estimating recruitment are described which are suitable for use where data are available on annual age composition, the numbers caught, and the effort expended in taking a known part of the catch.

The recruitment rate is estimated, as the proportion of new recruits in the recruited population, from age composition data year by year without regard to the sizes of the parent populations, and from the approximate fishing mortality rate.

The "q" population method estimates q, the coefficient of catchability, by comparing the rate of disappearance of a fixed group of year-classes with the rate of accumulation of fishing effort, using the rate of disappearance with age in a similar, unexploited population to eliminate the effects of natural mortality. The annual populations are then estimated from q and the catch per unit effort.

The natural mortality and reproduction method is basically a modification of the DeLury method with an allowance for natural reproduction and recruitment. It obtains a series of estimates of the base year population by considering each of the periods beginning in this year and ending in each successive subsequent year, the final estimate being the mean of this series.

The expected catch method takes as unknowns the initial population and the value of q, estimates the expected catches in each year, and compares them with the actual catches. It determines the values of the initial population and q which minimize the sum of the squares of the differences.

Examples are worked out in full for all methods.

INTRODUCTION

A great many methods have been developed in the last 40 years for estimating the size of animal populations. The majority probably fall into three main categories: (a) the estimation of density by direct sampling and combining this with information on the distribution and area covered by the population, (b) mark and recapture techniques, (c) measurement of relative changes of abundance by observation of presumably related quantities such as catch per unit effort, and relating these changes to the removal of known quantities, e.g. by fishing or hunting. In the field of fisheries, methods of the last two categories have been widely used, those of the last category being particularly extensively developed.

The present paper describes some methods in this category which have been worked out in connection with the study of baleen whale stocks, particularly in the North Pacific area. Whaling, although concerned with mammals, more closely resembles fishing in its essential features than it does most forms

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of terrestrial hunting. The animals sought inhabit an environment which has few permanent small-scale local features, which permits them to move freely in all directions, and which gravely limits the amount of direct observation which is possible. This has the effect of reducing the catching process to one which has many of the features of random sampling, despite the effort devoted to the production of detection techniques, and it is this feature which provides the basis for the widely-used hypothesis that the catch per unit effort is proportional to the size or density of the population. This hypothesis does not, however, always provide a satisfactory basis, and failure is probably often due to the nonuniform distribution of the fish and the efforts of the fishermen to work in places where they will get the best catch. Paloheimo and Dickie (1964) have shown that where fish are concentrated in schools the catch per unit effort may not be independent of the amount of effort and thus does not provide a satisfactory index of abundance. They have also shown, however, that under these conditions catch per unit effort is least satisfactory as an index when effort is measured as actual catching time, and less unsatisfactory if searching time is included. In studies of whaling operations up to the present the effort has been measured as the total operational time of the catching boats including searching and catching, as well as some towing time, and it has been assumed that catch per unit effort is directly proportional to abundance. The success of forecasts, based on these methods, of the size of some whale catches in the Antarctic (Holt and Gulland, 1965) suggests that this assumption provides a reasonably satisfactory basis for the estimation of whale stocks, although improvements would undoubtedly be possible. This hypothesis has therefore been adopted in the methods described here.

The other data used in these methods are derived in various ways from estimates of the age composition of the catch in each year. It is assumed that above a certain age this is representative of the age structure of the population, and that recruitment is progressive over several earlier years.

A feature of the North Pacific data, which was not so marked in the Antarctic studies, was the degree of variability particularly in the catch per unit effort. This was due both to random effects operating on much smaller efforts and catches, and to, in some instances, the exploratory nature of the fishery as new areas were continually being developed. In some parts of the region also, operations have only recently begun and very short series were available. All the methods described here have been developed with this situation in mind, and are designed to provide useful estimates even when there is considerable variability in the data.

The methods to be described, although developed in connection with studies of whale populations, are, of course, equally applicable to populations of other animals where the available data include the age composition year by year and numbers caught and the effort expended in taking a known part of the catch.

ESTIMATION OF RECRUITMENT

The majority of these methods require an estimate of the amount, or proportion, added to the stock each year by recruitment. In previous studies of Antarctic whale stocks (International Commission on Whaling, 1964) it was assumed that this formed a fixed proportion of the parent stock, which was taken to be the estimated number of mature females a certain number of years earlier. This method, while it produced useful results, did not allow either for any effects of changing population density on reproduction or on the survival of young whales, or for changes due to variation in the minimum size at which whales were actually being taken, as distinct from changes in the minimum legal size.

It was desirable, therefore, to attempt to develop a method which would enable a separate estimate to be made each year of the proportion of new recruits in that part of the population which is being exploited. The following method provides such an estimate, using data derived from the age composition of the exploited stock and approximate knowledge of the instantaneous rate of fishing mortality.

If $P_{1,a}$ = proportion in the catch of the a^{th} age group (partly recruited) in year 1.

$P_{2,a+1}$ = proportion in the catch of the $a+1^{th}$ age group in year 2.

$Q_{1,r}$ = proportion in the catch of all fully recruited age-groups from r up in year 1.

$Q_{2,r+1}$ = proportion in the catch of all age-groups from $r+1$ up in year 2.

M_1 = natural mortality rate of the unexploited part of the stock in year 1.

Z_1 = total mortality rate of new recruits in year 1.

Z_1' = total mortality rate of fully recruited part of the stock in year 1.

Z_2 = total mortality rate of new recruits in year 2.

Z_2' = total mortality rates of fully recruited part of the stock in year 2.

N_a = size of the a^{th} age-group at the beginning of year 1.

N_r = total size of all age-groups from r up at the beginning of year 1.

$U_{1,a}$ = proportion of the a^{th} age-group which is recruited in year 1.

$U_{2,a+1}$ = proportion of the $a+1^{th}$ age-group which is recruited in year 2.

Then we have for the year-class aged a in year 1:

Number at beginning of year 1 = N_a

Average number of recruits during year 1 = $N_a \cdot U_{1,a} \left[\frac{1 - e^{-Z_1}}{Z_1} \right]$

Number at beginning of year 2 = $N_a \left[U_{1,a}\, e^{-Z_1} + (1 - U_{1,a})\, e^{-M_1} \right]$

Average number of recruits during year 2

$$= N_a \cdot U_{2,a+1} \left[U_{1,a}\, e^{-Z_1} + (1 - U_{1,a})\, e^{-M_1} \right] \left(\frac{1 - e^{-Z_2}}{Z_2} \right)$$

Similarly for the group of fully recruited year classes aged r and up in year 1:

Number at beginning of year 1 = N_r

Average number during year 1 = $N_r \left[\frac{1 - e^{-Z'_1}}{Z'_1} \right]$

Number at beginning of year 2 = $N_r\, e^{-Z'_1}$

Average number during year 2 = $N_r\, e^{-Z'_1} \left[\frac{1 - e^{-Z'_2}}{Z'_2} \right]$

Then for year 1

$$\frac{\text{Average number of recruits in given year-class}}{\text{Average number in given fully recruited year-classes}} =$$

$$\frac{N_a \cdot U_{1,a} \left[\frac{1 - e^{-Z_1}}{Z_1} \right]}{N_r \left[\frac{1 - e^{-Z'_1}}{Z'_1} \right]} = \frac{P_{1,a}}{Q_{1,r}}$$

and for year 2

$$\frac{\text{Average number of recruits in same year-class}}{\text{Average number in same fully recruited year-classes}} =$$

$$\frac{N_a \cdot U_{2,a+1} \left[U_{1,a}\, e^{-Z_1} + (1 - U_{1,a})\, e^{-M_1} \right] \left(\frac{1 - e^{-Z_2}}{Z_2} \right)}{N_r\, e^{-Z'_1} \left[\frac{1 - e^{-Z'_2}}{Z'_2} \right]} = \frac{P_{2,a+1}}{Q_{2,r+1}}$$

Then putting $B = \frac{P_{2,a+1} \cdot Q_{1,r}}{P_{1,a} \cdot Q_{2,r+1}}$

we get

$$B = \frac{U_{2,a+1}\left[U_{1,a}\, e^{-Z_1} + (1 - U_{1,a})\, e^{-M_1}\right]\left[\frac{1 - e^{-Z_2}}{Z_2}\right]\left[\frac{1 - e^{-Z_1'}}{Z_1'}\right]}{U_{1,a}\, e^{-Z_1'}\left[\frac{1 - e^{-Z_1}}{Z_1}\right]\left[\frac{1 - e^{-Z_2'}}{Z_2'}\right]}$$

For practical purposes it may be assumed that even though the values of Z may vary considerably from year to year, it is unlikely that the relation between the fishing mortality rates for new recruits and older animals will change appreciably from one year to the next, and therefore, approximately, $\frac{Z_1}{Z_1'} = \frac{Z_2}{Z_2'}$.

The terms of the form $\left(\frac{1 - e^{-Z}}{Z}\right)$ may therefore be cancelled out, leaving

$$B = \frac{U_{2,a+1}\left[U_{1,a}\, e^{-Z_1} + (1 - U_{1,a})\, e^{-M_1}\right]}{U_{1,a}\, e^{-Z_1'}}$$

$$= U_{2,a+1}\, e^{-(Z_1 - Z_1')}\left[1 + \left(\frac{1}{U_{1,a}} - 1\right) e^{F_1}\right] \ldots\ldots (1)$$

writing $T = e^{-(Z_1 - Z_1')}$

we get

$$U_{1,a} = \frac{e^{F_1}}{\frac{B}{TU_{2,a+1}} - 1 + e^{F_1}} \qquad \ldots\ldots (2)$$

The values of B for the relevant age-groups in each year can be computed from the age distribution data, and do not involve any estimate of abundance. Thus, if an estimate of F_1 is available the relation between $U_{1,a}$ and $U_{2,a+1}$ can be calculated. Taking a value of 1.0 for $U_{2,a+1}$ for a year-class which can be assumed to be fully recruited in the year following the last year of the series for which data are available, and making some assumption as to the relation for each age-group between the values of U for the last year of the data and for the hypothetical following year, the values of U can be estimated successively for age-groups of diminishing age until a value U = 0 is reached. When a series of values of U has been constructed in this way for the final year, similar series can be constructed successively for earlier years using in each case the values already found for the next following year.

For most practical purposes it is probably satisfactory to take T = 1.0 in calculating U, but a means of obtaining an approximate estimate is available if the mortality rate of the youngest fully recruited year-class can be acceptable as an estimate of the mortality rate of the recruited part of the partially recruited year-classes. In this case an estimate of e^{-Z_1} is given by $\frac{P_{2,r+1}\phi_2}{P_{1,r}\phi_1}$ where $P_{1,r}$ and $P_{2,r+1}$ are the proportions in the catch in years 1 and 2 of the year-class which was first fully recruited in year 1 at age 0, and ϕ_1 and ϕ_2 are the catch per unit effort for the entire stock in years 1 and 2. An estimate of $e^{-Z_1'}$ is given by $\frac{Q_{2,r+1}\phi_2}{Q_{1,r}\phi_1}$ where the notation is as before.

Then

$$\hat{T} = \frac{P_{2,r+1} \cdot Q_{1,r}}{P_{1,r} \cdot Q_{2,r+1}} \quad \ldots\ldots (3)$$

The relative insensitivity of the estimates of U to the value used for F can be illustrated by the following table showing values of $U_{1,a}$ for various combinations of F and $B/TU_{2,a+1}$:

$\frac{B}{TU_{2,a+1}}$	F_1			
	.10	.25	.50	1.00
1.20	.847	.865	.892	.931
2.00	.525	.562	.622	.731
4.00	.269	.300	.355	.475

It is evident that, particularly at high values of $U_{1,a}$, large changes in F_1 have small effects on the estimate of $U_{1,a}$.

Before the results of this analysis of recruitment can be applied to the estimation of population they must be converted to give an estimate of the proportion of new recruits in the recruited part of the year-class for each age and year. This conversion can be made quite simply.

At the beginning of year 1 the number of recruits = $N_a U_{1,a}$

At the beginning of year 2 the survivors from these recruits equals $N_a \cdot U_{1,a} e^{-Z_1}$

At the beginning of year 2 the total number of recruits equals

$$N_a [U_{1,a} e^{-Z_1} + (1 - U_{1,a}) e^{-M_1}] U_{2,a+1}$$

Therefore, in year 2 the proportion of new recruits in the total recruits equals

$$R_{2,a+1} = 1 - \frac{U_{1,a}}{U_{2,a+1}} \cdot \frac{1}{U_{1,a} + (1 - U_{1,a})\ e^{F_1}} \quad \ldots\ldots (4)$$

In this calculation also, knowledge of F_1 is required, but here again the results are relatively insensitive to quite large changes in F_1, particularly at the higher values of $U_{1,a}$. This is illustrated by the following table which gives the value of $\frac{U_{1,a}}{U_{1,a} + (1 - U_{1,a})\ e^{F_1}}$ for various values of $U_{1,a}$ and F_1.

	F_1			
$U_{1,a}$	.10	.25	.50	1.00
.25	.232	.206	.168	.109
.50	.475	.438	.378	.269
.75	.731	.700	.645	.525
.90	.891	.875	.845	.768

To obtain the total proportion of new recruits in the exploited stock for any year (W_t) it is only necessary to sum the products of the proportion in the catch of each partially exploited year-class and the appropriate value of R. Thus,

$$W_t = \sum_{i=0}^{i=r-1} R_{t,i}\ P_{t,i} \quad \ldots\ldots (5)$$

where r is the youngest fully exploited year-class.

POPULATION ESTIMATES

The "q" Method

The basic assumption of this method is that the ratio between the instantaneous fishing mortality rate and fishing effort (q) is constant and it makes use of the pooled data for as many years as possible to obtain an estimate of q; it then computes the population for each year by dividing the estimated q into the catch per unit effort for the year. The method requires one or more base years in which it can be assumed that the population structure has been determined by natural mortality and that, in building up this population, recruitment has not varied from year to year.

The essential features of the method are:

(1) A single age-group is followed throughout to avoid recruitment effects.

(2) The relative abundance of the selected age-group is compared at each age with that of the group of the same age in years when the age structure was as yet unaffected by exploitation. This avoids the need for obtaining an estimate of M or even of assuming that it is independent of age.

(3) Estimates of total fishing mortality and of F are obtained for increasing periods of years so as to reduce to a minimum the effects of seasonal fluctuations, and are compared with the total effort over the same periods to obtain an estimate of q.

(4) Starting from a single base year, estimates of q are obtained for periods of increasing length and a weighted mean value obtained to further reduce the effects of variations in catch per unit effort from season to season.

The basic equation employed in this method may be written

$$\Sigma F_t = ln \left[\frac{Q_{1,r}}{Q_{t,r+t-1}} \cdot \frac{Q_{0,r+t-1}}{Q_{0,r}} \cdot \frac{\phi_1}{\phi_t} \right] \quad \ldots\ldots (6)$$

where F_t = total instantaneous fishing mortality from year 1 to year t.

$Q_{1,r}$ = proportion in the catch in year 1 of whales of age r (the first fully recruited age) and older.

$Q_{t,r+t-1}$ = proportion in the catch in year t of the same age-groups.

$Q_{0,r}$ = proportion in the catch from the previously unexploited stock of whales of age r and older.

$Q_{0,r+t-1}$ = proportion in the same year of whales age $r+t-1$ and older.

ϕ_1 = catch per unit effort in year 1.

ϕ_t = catch per unit effort in year t.

As the estimate of F obtained in this way refers to the decrease from the average population level in one season to the average level in a later one, the effort which has brought it about may be written

$$\Sigma X_t = 1/2 \left[X_1 + 2X_2 - - - + 2X_{t-1} + X_t \right] \quad \ldots\ldots (7)$$

An estimate of q can then be obtained as

$$q = \frac{\Sigma F_t}{\Sigma X_t}$$

When data are available for a number of years a series of estimates of q_t can be obtained by extending to successive years from the base year, and the mean of these taken as the value for the area. More weight should be given to the estimates obtained over the longer periods, since the random errors will tend to be proportionately smaller. The estimate of q which should be employed is therefore:

$$\bar{q} = \frac{\sum_{t=2}^{T} \Sigma F_t}{\sum_{t=2}^{T} \Sigma X_t} \quad \ldots\ldots (8)$$

where T is the total number of years available in the series.

An estimate of the mid-season recruited population in year t is then given by

$$N_t = \frac{\phi_t}{\bar{q}} \quad \ldots\ldots (9)$$

From Estimates of Natural Mortality and Reproduction

The previous method made use of the estimated age structure of the populations in successive years. An alternative method which does not require detailed data of this nature but calls for data on catch and effort and generalized estimates of reproduction and natural mortality coefficients was developed in the studies of the Antarctic stocks as a modification of the DeLury method (International Commission on Whaling, 1964, p. 54). The method was there employed to yield a single estimate of the population at the beginning of the period studied, by combining data for the full series of years available. This estimate is, however, particularly sensitive to the relation between the catch per effort (C/E) in the first and last years of the period. A better estimate is obtained for the initial population by extending the original method to obtain a series of estimates for the successive periods of years all beginning with the same year and increasing up to the total period available. The final estimate is then obtained as a mean of this series.

The generalized formula developed from that given in the work referred to then becomes

$$N_1 = \frac{\sum_{1}^{t-1} C_i}{1 - Y_t - M \sum_{1}^{t-1} Y_i + r \sum_{2-n}^{t-n} Y_i} \quad \ldots\ldots (10)$$

where N_1 = recruited population at the beginning of year 1.

C_i = catch in year i.

Y_i = C/E in year i / C/E in year 1.

M = annual natural mortality rate.

r = ratio of number of recruits to number of parent stock.

n = mean age at recruitment.

In applying this method, a series of estimates of N_1 may be obtained by using increasing values of t up to the maximum available. Thus, for the first estimate $t = 2$ and Y_t refers to the year following year 1; for the next estimate $t = 3$ and Y_t refers to the second year after year 1; and so on. When the full series of estimates has been obtained in this way, the mean value is calculated. If desired a similar series of estimates can be obtained for each subsequent year up to the last but one; the number of estimates in the series decreases by one for each successive year.

As originally employed, or in the form of equation 10 with the variables as defined above, the assumption is made that the catch per unit effort in each season is proportional to the size of the stock at the beginning of the year. It is probably better to assume that the catch per unit effort is proportional to the stock at mid season; this does not involve any change in equation 10 if N_1 and C_i are redefined so that

N_1 = recruited population at mid point of year 1

and $C_i = 1/2$ (catch in year i + catch in year $i+1$).

In this form it is also assumed that recruitment is a constant proportion of the parent stock n years earlier. If, however, the proportion of new recruits in the recruited stock in each year is known (W_i), this information may be used instead, and the equation then becomes

$$N_1 = \frac{\sum_{1}^{t-1} C_i}{1 - Y_t - M \sum_{1}^{t-1} Y_i + \sum_{2}^{t} W_i Y_i} \qquad \ldots\ldots (11)$$

By Comparison of Actual and Expected Catches

The basis of this method is to obtain an estimate which minimizes the sum of the squares of the differences between actual and expected catches. The unknown parameters are the initial population size (N_1) and the ratio between instantaneous fishing mortality rate and fishing effort (q). A constant instantaneous natural mortality rate (M) is assumed, and recruitment is obtained by the method described in the first part of this paper. The method proceeds by constructing the populations in successive years by taking the initial population, subtracting the actual catch, subtracting natural mortality (by multiplying by e^{-M}), adding recruitment (by dividing by (1–W) where W is here the proportion of new recruits in the *next* year), and repeating this process successively, using each estimate so obtained as the initial population for the following year. The expected catches are then obtained for each year by dividing the estimated average population during the season by the estimated fishing mortality rate (q times the known effort). To simplify computation the average population is taken to be the initial population less half the catch;

provided that F is less than .35 the error in the expected catch introduced by this approximation is less than 1%, and can be considered negligible in view of the other irregularities in the data. It will be noted that the method assumes that the processes of catching, natural mortality, and recruitment proceed successively and not simultaneously. This greatly simplifies the calculations, and probably does not introduce major errors, particularly for seasonal fisheries such as whaling.

Following the method as outlined above, and taking C_t and X_t to be the catch and effort respectively in year t, we have

Year 1 Initial population $= N_1$

Survival to beginning of next season $= (N_1 - C_1)\, e^{-M}$

Expected catch $= \left(N_1 - \frac{C_1}{2}\right) qX_1$

Year 2 Initial population $= N_2$ $= \frac{(N_1 - C_1)\, e^{-M}}{1-W_2}$

Survival to beginning of next season $= \left[\frac{(N_1 - C_1)\, e^{-M}}{1-W_2} - C_2\right] e^{-M}$

Expected catch $= \left[\frac{(N_1 - C_1)\, e^{-M}}{1-W_2} - \frac{C_2}{2}\right] qX_2$

Continuing in this way we can show that at the beginning of year t the population equals

$$N_t = \frac{e^{-(t-1)M}}{\prod_{i=2}^{t}(1-W_i)}\left[N_1 - C_1 - \frac{C_2(1-W_2)}{e^{-M}} \cdots \frac{C_i \prod_{j=2}^{i}(1-W_j)}{e^{-(i-1)M}} \cdots \frac{C_{t-1}\prod_{j=2}^{t-1}(1-W_j)}{e^{-(t-2)M}}\right]$$

$$= A_t\left[N_1 - f(C)_{t-1}\right] \quad \ldots\ldots (12),$$

where $A_t = \frac{e^{-(t-1)M}}{\prod_{i=2}^{t}(1-W_i)}$

and $f(C)_{t-1} = C_1 + \sum_{i=2}^{t-1}\frac{C_i}{A_i}$

In year t also the difference between actual and expected catches is given by

$$C_t - \left(N_t - \frac{C_t}{2}\right) qX_t = C_t\left[1 + \frac{qX_t}{2}\right] - qX_tN_t \quad \ldots\ldots (13)$$

The sum of the squares of the differences for the period year 1 to year T equals

$$\sum_{t=1}^{T}\left[C_t\left(1+\frac{qX_t}{2}\right)-qX_tN_t\right]^2=d^2 \qquad \ldots\ldots(14)$$

Expanding and grouping like terms we can obtain

$$d^2=K+Lq+PN_1q+RN_1q^2+SN_1^2q^2+Uq^2 \qquad \ldots\ldots(15)$$

where $K=\Sigma C_t^2$; $L=\Sigma C_t^2X_t+2\Sigma C_tX_tA_tf(C)_{t-1}$; $P=-2\Sigma C_tX_tA_t$;

$$R=-[\Sigma C_tX_t^2A_t+2\Sigma X_t^2A_t^2f(C)_{t-1}];\ S=\Sigma X_t^2A_t^2$$

$$U=\frac{1}{4}\Sigma C_t^2X_t^2+\Sigma C_tX_t^2A_tf(C)_{t-1}+\Sigma X_t^2A_t^2f^2(C)_{t-1}$$

where all terms are summed from $t=1$ to $t=T$.
Differentiating with respect to N_1 and q, and setting equal to 0, gives the following normal equations which define the conditions for which the sum of squares is a minimum:

$$D_{(N_1)}=Pq+Rq^2+2SN_1q=0 \qquad \ldots\ldots(16)$$

$$D_{(q)}=L+2Uq+PN_1+2RN_1q+2SN_1^2q=0 \qquad \ldots\ldots(17)$$

Solving for N_1 and q gives

$$N_1=\frac{LR-2PU}{RP-2SL} \qquad \ldots\ldots(18)$$

$$q=\frac{RP-2SL}{4SU-R^2} \qquad \ldots\ldots(19)$$

When N_1 has been estimated, the populations for subsequent years can be estimated from equation 12

$$N_t=A_t[N_1-f(C)_{t-1}]$$

In obtaining estimates satisfying the condition that the sum of the squares of the differences between actual and expected catches is minimized C_t, X_t, and W_t are provided by the basic data, and the terms A_t and $f(C)_{t-1}$ are calculated from them using an arbitrarily selected value of M. The sums L, P, R, S, and U can then be easily computed.

The calculations are not laborious for short series or if a high-speed computer is available, and it is relatively simple to try the effect of inserting various values of M.

METHODS OF CALCULATION

Use of these methods may be illustrated by application to a short series of data for the fin whale stock in Area III of the North Pacific, between 140 and 160°W. The relevant catch and effort statistics are set out in Table I, effort being in

$$\frac{\text{Catcher's day's work} \times \text{catcher tonnage}}{1000}.$$

The age composition of the males in each year's catch is shown in Table II. Ages were determined by examination of the laminations in the ear plugs on the assumption that two laminations are formed each year. Age compositions were calculated by applying to the length distribution in each year an age length key based on material collected over a range of years in the waters of the North Pacific east of 180°W. In all calculations whales of age 6 are taken to be fully recruited.

Population Estimate by q Method

This estimate is obtained before that of recruitment since it does not require any estimate of recruitment, and can be used to provide the estimate of F for each year which is needed for the recruitment calculations. The calculations for the males are set out in Table III, yielding finally a value for $\bar{q}$ of .000907. Similarly, a value for the females of .001028 is obtained, giving a pooled mean of .000968.

The pooled mean is then used to obtain the estimates of the total population and of F, as shown in Table IV, the population being given by $\frac{\phi}{\bar{q}}$ and F by $\bar{q}X$.

Recruitment

The first step in the calculation of recruitment is construction of a table of B.

Table I. Catch and effort statistics for fin whales in Area III in the North Pacific Ocean.*

		Catch			Catch per unit effort			Relative C/E
Year	Effort	Male	Female	Total	Male	Female	Total	Total
1961	158	210	215	425	1.329	1.361	2.690	1.000
1962	214	321	279	600	1.502	1.306	2.808	1.044
1963	486	514	528	1042	1.049	1.095	2.144	.798
1964	333	297	249	546	.892	.748	1.640	.610

TABLE II. Age composition of male fin whales caught in Area III.

Age	Year			
	1961	1962	1963	1964
0	.0000	.0000	.0000	.0000
1	.0008	.0027	.0050	.0041
2	.0103	.0346	.0565	.0455
3	.0470	.0825	.1099	.0944
4	.0363	.0849	.1030	.0856
5	.0386	.0743	.0812	.0711
6	.0379	.0711	.0688	.0676
7	.0370	.0586	.0523	.0531
8	.0545	.0586	.0522	.0553
9	.0754	.0567	.0512	.0566
10	.0440	.0531	.0484	.0508
11	.0280	.0307	.0251	.0292
12	.0341	.0420	.0358	.0373
13	.0575	.0418	.0385	.0436
14	.0389	.0398	.0339	.0362
15	.0457	.0356	.0303	.0369
16	.0629	.0373	.0340	.0380
17	.0420	.0305	.0275	.0318
18	.0429	.0320	.0266	.0320
19	.1235	.0221	.0197	.0205
20	.0186	.0154	.0129	.0143
over 20	.1240	.0958	.0881	.0963

TABLE III. Estimation of q for male fin whales in Area III.

Year	ΣX	R	ϕ_1/ϕ_2	$R \times \phi_1/\phi_2$	ΣF
1961	0	1.0000	1.000	1.000	0
1962	186	1.2755	.8847	1.128	.121
1963	536	1.5108	1.2668	1.914	.649
1964	945	1.4095	1.4902	2.099	.742
	1667				1.512

$$\bar{q} = \frac{1.512}{1667} = .000907$$

Taking 1961 as year 1 and 1962 as year 2,

for $a = 0$ $$B = \frac{.0027 \times .8669}{0 \times .6500} = \infty$$

where .8669 and .6500 are the cumulative sums from age 6 upwards in 1961 and from age 7 in 1962, respectively;

TABLE IV. Estimation of total fin whale population for Area III by the q method.

Year	Catch/Effort	Population	Effort	F
1961	2.690	2779	158	.153
1962	2.808	2902	214	.207
1963	2.144	2215	486	.470
1964	1.640	1694	333	.322

for $a = 1$ $$B = \frac{.0346 \times .8669}{.0008 \times .6500} = 56.983$$

$a = 2$ $$B = \frac{.0825 \times .8669}{.0103 \times .6500} = 10.651.$$

The values of B obtained in this way are given in column 3 of Table V.

In computing the values for 1964, no values of P and Q for 1965 were available, it has therefore been assumed, in order to get estimates of B, that they would be the same as those for 1964.

Above the age of full recruitment the most probable value of B is 1.0, and fluctuations about this value will occur due to sampling errors. In the present calculations it has been assumed that this level has been reached at the second age at which B falls below 1.0, and the values marked with an asterisk have been treated as 1.0 in subsequent calculations.

The next quantity to be calculated is U, the proportion of recruits in each age-group. This requires knowledge of T, which is taken as 1.0 in this example, and of F, which has been obtained from the previous population estimate and is set out in the last column of Table IV. Calculations have to be started with a fully recruited year-class (for which U = 1.0) in the last year of the series. As in the calculation of B, it is necessary to assume that the same values would occur for 1965 as occur in the 1964 data. Therefore, starting in 1964, when F = .322, $e^F = 1.3799$, we have

$$U_{64,5} = \frac{e^{F_{64}}}{\frac{B}{TU_{64,6}} - 1.0 + e^{F_{64}}} = \frac{1.3799}{\frac{1.0519}{1.0 \times 1.0} - 1.0 + 1.3799} = .9637.$$

Then

$$U_{64,4} = \frac{1.3799}{\frac{.9201}{1.0 \times .9637} - 1.0 + 1.3799} = 1.0340$$

TABLE V. Computation of recruitment rate for fin whales in Area III; the third to sixth columns show the detailed calculations for males, and the last column the combined mean proportion of new recruits of all ages for both sexes.

Year	Age	B	U	R	W	Combined mean
1961	0	∞	0	0	0	
	1	56.9833	.0060	0	0	
	2	10.6507	.0729	0	0	
	3	2.4085	.3936	0	0	
	4	2.7263	.3595	0	0	
	5	2.4538	.4449	0	0	
	6	2.0634	1.0000	0	0	
	7	2.1139	1.0000	0	0	
	8	1.3887	1.0000	0	0	
					0	0
1962	0	∞	0	–	–	
	1	25.3709	.0309	1.0000	.0027	
	2	3.9742	.2925	.9824	.0340	
	3	1.5606	.6731	.9061	.0748	
	4	1.1972	.8618	.5848	.0496	
	5	1.1577	.8863	.6332	.0470	
	6	.9197	1.0000	.5925	.0421	
	7	1.1144	1.0000	0	0	
	8	1.0937	1.0000	0	0	
					.2502	.2113
1963	0	∞	0	0	0	
	1	9.2416	.0863	.0000	.0050	
	2	1.7349	.6840	.9606	.0534	
	3	.7951	1.0000	.7484	.0823	
	4	.7050*	.9770	.3592	.0370	
	5	.8501*	1.0000	.1647	.0134	
	6	.7982*	1.0000	.1362	.0094	
	7	1.0818*	1.0000	0	0	
	8	1.1073*	1.0000	0	0	
					.2004	.2229
1964	0	∞	0	0	0	
	1	12.2673	.0570	1.0000	.0041	
	2	2.2950	.5148	.8918	.0406	
	3	1.0035	.9975	.4236	.0400	
	4	.9201	1.0000	0	0	
	5	1.0519	.9637	0	0	
	6	.8694*	1.0000	0	0	
	7	1.1525*	1.0000	0	0	
	8	1.1327*	1.0000	0	0	
					.0847	.0806

but the recruited proportion of a year-class cannot exceed 1.0 and therefore we put $U_{64,4} = 1.0000$. Then

$$U_{64,3} = \frac{1.3799}{\frac{1.0035}{1.0 \times 1.0} - 1.0 + 1.3799} = .9975$$

$$U_{64,2} = \frac{1.3799}{\frac{2.2950}{1.0 \times .9975} - 1.0 + 1.3799} = .5148$$

$$U_{64,1} = \frac{1.3799}{\frac{12.2673}{1.0 \times .5148} - 1.0 + 1.3799} = .0570$$

$$U_{64,0} = \frac{1.3799}{\frac{\infty}{1.0 \times .0567} - 1.0 + 1.3799} = 0.$$

Calculation then proceeds to 1963 using the 1964 values of U in the denominator, and then successively to 1962, and 1961. The resulting values are shown in Table V, those greater than 1.0 as originally calculated having been converted to 1.0.

The next step is the calculation of R, the proportion of new recruits in the recruited part of each year-class. This is obtained from equation 4. In the first year the entire recruited stock are virtually new recruits and it would be meaningless to attempt to estimate a proportion. Calculations may therefore be started for the second age-group in the first year of the series with $F = .153$, $e^F = 1.1653$, and we get

$$R_{62,1} = 1 - \frac{0}{0 + (1-0)1.1653} = 1.0$$

$$R_{62,2} = 1 - \frac{.0060}{.2925(.0060 + (1 - .0060)1.1653)} = .9824$$

$$R_{62,3} = 1 - \frac{.0729}{.7004(.0729 + (1 - .0729)1.1653)} = .9061$$

$$R_{62,4} = 1 - \frac{.3936}{.8618(.3936 + (1 - .3936)1.1653)} = .5848$$

and so on for older age-groups and subsequent years. The complete results are again included in Table V.

Finally, the proportion of new recruits in the total recruited stock is obtained by multiplying the proportion of each year-class by the proportion of new recruits in it, and summing. Thus, for 1962 males we have

Age class	R	Proportion	W
0	–	0	0
1	1.0000	.0027	.0027
2	.9824	.0346	.0340
3	.9061	.0825	.0748
4	.5848	.0849	.0496
5	.6332	.0743	.0470
6	.5925	.0711	.0421
Total proportion:			.2502

The complete results are again shown in Table V. The last column shows the mean totals for males and females in each year.

Population Estimate by Natural Mortality and Reproduction Method

By means of equation 11 estimates of the 1961 population can be obtained, using first only the range 1961–62, then by extending to 1963, and finally to 1964. Then, taking M = .08, the first estimate is

$$N_1 = \frac{512.5}{1 - 1.044 - .08(1.000) + (.211 \times 1.044)} = 5312.$$

Extending to 1963

$$N_1 = \frac{1333.5}{1 - .798 - .08(2.044) + (.211 \times 1.044 + .223 \times .798)} = 3047.$$

and to 1964

$$N_1 = \frac{2127.5}{1 - .610 - .08(2.842) + (.211 \times 1.044 + .223 \times .798 + .081 \times .610)} = 3484.$$

The mean of the three estimates is 3948.

Similarly, estimates for the 1962 stock are 2513 for range to 1963 and 3280 with range to 1964, the mean being 2896.

The single estimate obtainable in the same way for 1963 is 3659.

No estimate can of course be obtained directly in this way for the 1964 stock.

Population Estimate by Comparison of Catch Method

This involves the computation of the successive values of the function A_t and $f(C)_{t-1}$ for each year of the series, and then calculations for the whole series of the various sums of products. From these, values of N_1 and q are calculated directly. For the first year of the series $t = 1$ and $W_i = 0$, so that $A_1 = 1.0$. The subsequent values are obtained by multiplying the preceding value by $\frac{e^{-M}}{1 - W_t}$. Thus, with $M = .08$, $e^{-M} = .9229$, and with values of W_t as given in Table V, we have A_t as shown in Table VI. $f(C)_t$ is obtained by summing the terms C_i/A_i for successive years, C_i being the catch as shown in Table I. Summing the various products gives values for the secondary expressions of

$$K = 1.9245 \times 10^6 \qquad R = -2.1078 \times 10^9$$

$$L = 3.0366 \times 10^9 \qquad S = 7.6013 \times 10^5$$

$$P = -2.3501 \times 10^6 \qquad U = 1.5824 \times 10^{12}$$

Substituting in equations 18 and 19 then gives for the population at the beginning of 1961

$$N_1 = 3076 \qquad \text{and} \qquad \hat{q} = .000915.$$

The populations in subsequent years can then be obtained from equation 12, e.g. for 1962

$$N_2 = 1.1704(3076 - 425) = 3103$$

Table VI. Computation of the intermediate quantities for the estimation of the fin whale population of Area III by the expected catch method.

Year	A_t	C_t/A_t	$f(C)_{t-1}$
1961	1.0000	425	0
1962	$\frac{1.0000 \times .9229}{.7887} = 1.1704$	513	425
1963	$\frac{1.1704 \times .9229}{.7771} = 1.3904$	749	938
1964	$\frac{1.3904 \times .9229}{.9194} = 1.3961$	391	1687

and for 1963

$$N_3 = 2974$$

and for 1964

$$N_4 = 1940.$$

If desired the expected catches can be most easily calculated as

$$C_t = \left(N_t - \frac{C_t}{2}\right) qX_t$$

so that for 1961 the expected catch is

$$C_1 = \left(3076 - \frac{425}{2}\right).000915 \times 158 = 413.9.$$

The sum of squares of differences can be obtained, if required, either through the expected catches by working out, squaring and summing the differences from the actual catches, or else directly from equation 15. In the present example, it is of interest to use the more detailed method, and we have

Year	*Actual catch*	*Expected catch*	*Difference*	*Difference squared*
1961	425	413.9	11.1	122.84
1962	600	547.8	52.2	2724.51
1963	1042	1090.3	−48.3	2331.07
1964	546	507.7	38.3	1469.18
	Sum of squares of differences:			6647.60

DISCUSSION

The following table compares the estimates of population for the various years:

Year	*q method*	*M and R method*	*Expected catch method*
1961	2779	3948	3076
1962	2902	2896	3103
1963	2215	3659	2974
1964	1694	–	1940

In this case there is a fairly good level of agreement between the estimates obtained by the three methods. The level of agreement is influenced by the value which is arbitrarily chosen for M in the second and third methods, although the estimates are not highly sensitive to this parameter. The estimates for the 1961 population for these two methods for three values of M are

M	*M and R method*	*Expected catch method*
.05	3261	2803
.08	3948	3076
.12	5700	3535

The expected catch method is apparently the less sensitive of the two methods.

This example represents a case in which exploitation apparently led to a rapid and fairly consistent decrease in the size of the stock, as measured by the catch per unit effort, and such data are favourable for these methods, as for most methods of population estimation based on relative changes in abundance.

As stated in the introduction to this paper, the methods were developed in an effort to find ways of dealing with rather erratic data. It is therefore interesting to examine the results obtained in a less favourable case. That chosen is the North Pacific fin whale stock for Area IV (160 to 180°W). The basic catch and effort data are summarized in Table VII. The age structure is generally not greatly unlike that for Area III given in Table II. In this case M has, however, been taken as .12, as this appears to correspond rather more closely to the age structure of the population at the beginning of exploitation than does .08.

TABLE VII. Catch and effort data for fin whales in Area IV in the North Pacific Ocean.

Year	Effort	Total catch	Total catch per unit effort
1954	204	752	3.6863
1955	279	1212	4.3441
1956	288	719	2.4965
1957	404	1101	2.7252
1958	324	894	2.7593
1959	256	743	2.8968
1960	400	1166	2.9145
1961	317	904	2.8552
1962	423	966	2.2829

The estimates obtained by all three methods are set out in Table VIII. In this case the results obtained by the second method are highly erratic, with some negative estimates actually occurring. This method seems therefore the least satisfactory of the three where the original data are irregular, due to high sampling errors or to a fishery which is not operating regularly over the whole stock. The other two methods give results which are consistent within themselves, but those for the q method are about 12% higher than those for the expected catch method.

Thus, for both sets of data, the estimates given by the q and expected catch methods differ only by amounts which could be eliminated by comparatively small changes in the value arbitrarily selected for the natural mortality rate in the latter method. The q method, in effect, assumes natural mortality rates equal to those producing the age structure that existed in the base year,

TABLE VIII. Comparison of estimates of the population of fin whales in Area IV obtained by the three methods.

Year	q method	M and R method	Expected catch method
1954	18162	10757	14357
1955	21403	10392	14777
1956	12300	−40105	13840
1957	13427	31793	12865
1958	13595	76151	12373
1959	14272	13991	11184
1960	14360	−8666	12244
1961	14067	4240	10502
1962	11248	–	10320

before exploitation took place. In deciding, therefore, which method to accept, when there is some discrepancy, a useful first step would be to examine the available data regarding natural mortality rates, and to study them in relation both to the value adopted in the expected catch method and to that implicit in the base population in the q method.

A minor discrepancy can arise from the fact that the first two methods estimate the mid season population, while the expected catch method estimates the initial population. The last method should, therefore, give slightly higher estimates than the other two, although the difference should only be of the order of half the annual catch.

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The Effect of Hemolysis Upon the Oxygen Affinity of Hemoglobin in the Atlantic (*Salmo salar*) and Landlocked Salmon (*Salmo salar sebago*)[1]

By Edgar C. Black[2], Harold H. Tucker[3], and Donald Kirkpatrick[4]

Department of Physiology
Dalhousie University, Halifax, N.S.

ABSTRACT

Confirming the work of other investigators, hemolysis was shown to diminish the influence exerted by carbon dioxide upon the affinity of oxygen for hemoglobin (Bohr effect). There was no consistent difference between blood taken from salmon acclimated to summer and winter conditions. Furthermore, there was no consistent difference between the effect of hemolysis on the Bohr effect between Atlantic and landlocked salmon.

INTRODUCTION

Krogh and Leitch (1919) first demonstrated the marked sensitivity of fish hemoglobin–oxygen equilibria to carbon dioxide (Bohr effect). Root (1931) observed that the hemoglobin in a series of experiments on marine fish possessed a very great Bohr effect. Black and Irving (1938) showed that the Bohr effect was obliterated in the hemoglobin of two freshwater fish when the erythrocytes were hemolysed either by saponin or oxalate. Root et al. (1939) showed that while hemolysis usually erased the marked Bohr effect in marine fish, this was not always the case. Benditt et al. (1941) observed that the Bohr effect of the blood of Atlantic salmon taken from brackish water was only influenced partially by hemolysis.

The present work was undertaken to confirm the work of Benditt et al. (1941) and to compare the effect of hemolysis in summer- and winter-acclimated Atlantic and landlocked salmon.

METHODS

Atlantic and landlocked salmon were supplied by the Fish Culture Branch of the Department of Fisheries, Ottawa, Canada. During the summer, the fish were held in rearing ponds at Grand Lake, Nova Scotia, and during the winter at Bedford, Nova Scotia. The summer temperatures at Grand Lake ranged from 5 to 20 C and winter temperatures at Bedford ranged from 0 to 22 C.

[1]Received for publication May 19, 1966.
[2]Present address (E.C.B.): Department of Physiology, University of British Columbia, Vancouver 8, B. C.
[3]Present address (H.H.T.): Department of Surgery, Dalhousie University, Halifax, N.S.
[4]Present address (D.K.): Kentville, N. S.

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In every instance, blood from a single salmon was used for the day's work. Half of each sample was hemolysed with dry powdered saponin. The analyses were carried out in the Department of Physiology, Dalhousie University. Procedures used are those described in papers by Black and Irving (1938), and Black et al. (1966a). All equilibrations were carried out at 15 C.

RESULTS AND DISCUSSION

Figure 1 portrays conventional oxygen dissociation curves for Atlantic salmon whole and hemolysed blood from a single fish acclimated to winter conditions at a $p\ CO_2$ of 10 mm Hg. These curves are compared with the average curve for whole blood at 0–1 mm Hg $p\ CO_2$ taken from Black et al. (1966b). It is clear that hemolysis reduced the Bohr effect to approximately one half of the same in whole blood. This effect was first demonstrated by Black and Irving (1938).

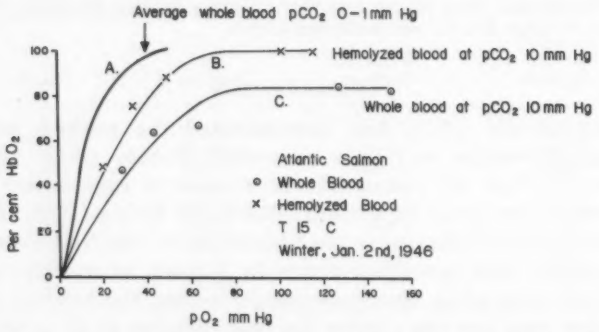

FIG. 1. Oxygen dissociation curves of whole and hemolysed blood of Atlantic salmon, acclimated to winter conditions and equilibrated at 15 C at $p\ CO_2$ of 10 mm Hg. Average curve for $p\ CO_2$ 0–1 mm Hg, taken from Black et al. (1966b) (January 2, 1946).

Figure 2, illustrates the effect of hemolysis upon the oxygen capacity of whole and hemolysed blood of Atlantic salmon acclimated to winter conditions at various tensions of carbon dioxide and at a $p\ O_2$ of 150–155 mm Hg. The curves can be compared with the conventional oxygen dissociation curves shown in Fig. 1 at two points only, viz. $p\ CO_2$ 0–1 mm Hg, and a p CO_2 of 10 mm Hg. The curves demonstrate that hemolysis has reduced the influence of carbon dioxide upon the oxygen affinity of hemoglobin. This method of representing data was used by Black and Irving in 1938. Figures 3 and 4 show similar data for blood of Atlantic salmon acclimated to summer conditions. There was no difference between the curves for hemolysed and whole blood in Fig. 3. However, in Fig. 4, hemolysis effected a marked reduction in the Bohr effect. No explanation is offered for the difference in the effect of hemolysis in Fig. 3 and 4. Benditt et al. (1941) also demonstrated reduction

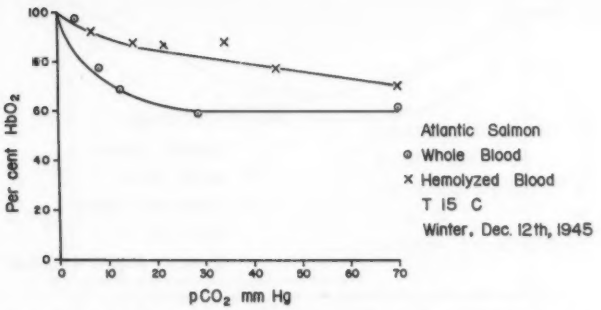

FIG. 2. Affinity of hemoglobin for oxygen in whole and hemolysed blood of Atlantic salmon acclimated to winter conditions and equilibrated at 15 C at $p\ O_2$ 150–155 mm Hg and at various tensions of carbon dioxide (December 12, 1945).

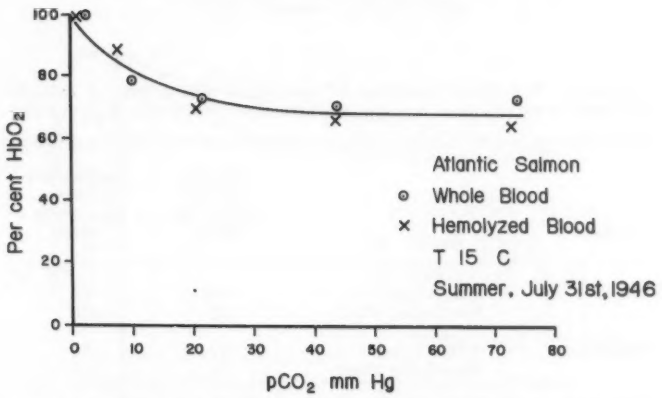

FIG. 3. Affinity of hemoglobin for oxygen in whole and hemolysed blood of Atlantic salmon acclimated to summer conditions and equilibrated at 15 C at $p\ O_2$ 150–155 mm Hg and at various tensions of carbon dioxide (July 31, 1946).

in the Bohr effect caused by hemolysis (Table I). Table I also includes data taken from Fig. 2–4 of this paper.

The influence of hemolysis upon the blood of landlocked salmon acclimated to winter conditions is illustrated in Fig. 5, and upon landlocked salmon acclimated to summer conditions in Fig. 6. The influence of hemolysis is also summarised in Table I. In general, the data for the landlocked salmon are concordant with those of the Atlantic salmon.

Hemolysis diminished the Bohr effect to the extent first described by Benditt et al. (1941) in all experiments except for those represented in Fig. 3.

Presumably, the intact erythrocyte provides a special environment favouring the Bohr effect beneficial in countering the adverse effect of lowered

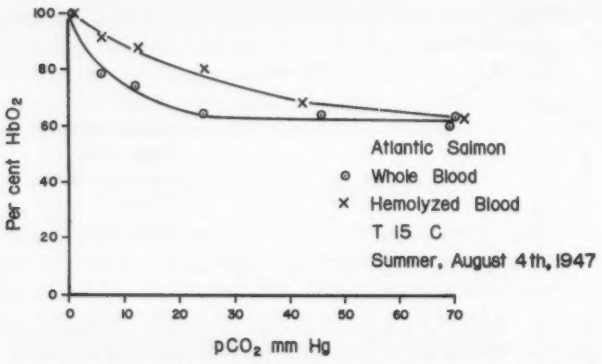

FIG. 4. Affinity of hemoglobin for oxygen in whole and hemolysed blood of Atlantic salmon acclimated to summer conditions and equilibrated at 15 C at $p\ O_2$ 150–155 mm Hg and at various tensions of carbon dioxide (August 4, 1947).

TABLE I. Percentage saturation of hemoglobin with oxygen of Atlantic and landlocked salmon at a partial pressure of carbon dioxide of 25 mm Hg.

Season		Atlantic salmon percentage HbO_2	Landlocked salmon percentage HbO_2
Winter	Whole blood	60	72
	Hemolysed blood	83	84
Summer	Whole blood	72	58
	Hemolysed blood	72	75
Summer	Whole blood	63	
	Hemolysed blood	87	
Summer	Whole blood	75	
	Hemolysed blood[a]	83	

[a]Data of Benditt et al., 1941.

temperature on the oxygen affinity of hemoglobin. Barcroft and Camis (1909) were the first to show the very great effect of various salts and salt concentration upon the equilibrium between oxygen and mammalian hemoglobin. Their data were confirmed and extended by Sidwell et al. (1938). Riggs (1960) has shown that the Bohr effect in mammalian hemoglobin is related to the sulfhydril groups. It is likely that the active groups are affected differently by the chemical environment provided by the intact cells as compared with the hemolysed solution.

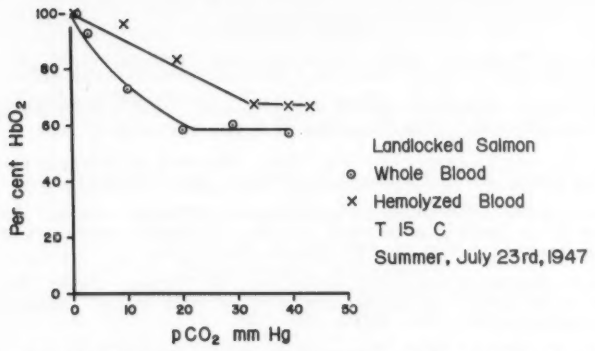

FIG. 5. Affinity of hemoglobin for oxygen in whole and hemolysed blood of landlocked salmon acclimated to winter conditions and equilibrated at 15 C at $p\ O_2$ 150–155 mm Hg and at various tensions of carbon dioxide (February 5, 1946).

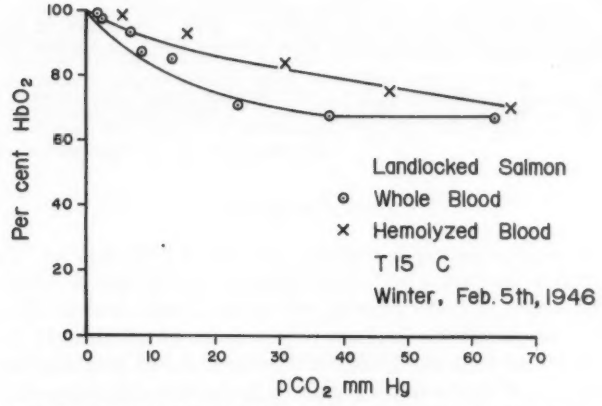

FIG. 6. Affinity of hemoglobin for oxygen in whole and hemolysed blood of landlocked salmon acclimated to summer conditions and equilibrated at 15 C at $p\ O_2$ 150–155 mm Hg and at various tensions of carbon dioxide (July 23, 1947).

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Oxygen Dissociation Curves of the Blood of Landlocked Salmon (*Salmo salar sebago*) Acclimated to Summer and Winter Temperatures[1]

By Edgar C. Black,[2] Donald Kirkpatrick,[3] and Harold H. Tucker[4]

Department of Physiology
Dalhousie University, Halifax, N.S.

ABSTRACT

Oxygen dissociation curves for the blood of landlocked salmon (*Salmo salar sebago*) were constructed at three tensions of carbon dioxide, viz. 0–1 mm Hg, 10 mm Hg, and 40 mm Hg; and at two temperatures: 5 C and 25 C. Carbon dioxide dissociation curves were constructed at 5 C. Gas tensions in mixed venous blood were determined for one salmon only, at 5 C. Oxygen dissociation curves at two temperatures were carried out for fish acclimated to summer and winter conditions.

Oxygen and carbon dioxide dissociation curves for the landlocked were similar in character to those of the Atlantic salmon (*Salmo salar*). The Bohr effect in the landlocked salmon is of sufficient magnitude to counterbalance the effect of lowered temperature upon the affinity of hemoglobin for oxygen. No influence of acclimation was observed for the effect of carbon dioxide on the equilibrium between oxygen and hemoglobin.

INTRODUCTION

Krogh and Leitch (1919), in their pioneer investigation of the respiratory characteristics of fish blood, showed that the increased sensitivity of fish hemoglobin to carbon dioxide offset the adverse effect of lowered temperature. Black et al. (1966a, b) demonstrated a marked sensitivity to carbon dioxide for the hemoglobin of brook trout (*Salvelinus fontinalis*) and Atlantic salmon. They also showed that acclimation to summer and winter temperatures exerted no effect on the equilibria between hemoglobin and oxygen.

The present investigation was undertaken to determine the respiratory characteristics of landlocked salmon, acclimated to summer and winter temperatures.

METHODS

The landlocked salmon were provided and cared for by the Fish Culture Branch of the Department of Fisheries, Ottawa, Canada. During the summer the fish were held at the salmon rearing station at Grand Lake, Nova Scotia,

[1]Received for publication May 16, 1966.
[2]Present address (E.C.B.): Department of Physiology, University of British Columbia, Vancouver 8, B. C.
[3]Present address (D.K.): Kentville, N.S.
[4]Present address (H.H.T.): Department of Surgery, Dalhousie University, Halifax, N.S.

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and transferred to the Bedford Hatchery in Bedford, Nova Scotia, for the winter temperatures. Both stations are operated by the Department of Fisheries. Water temperatures at the Bedford Hatchery during the fall to spring ranged from 0 to 22 C, while the temperatures at Grand Lake during the spring and summer were between 5 and 20 C.

Materials and procedures for taking blood samples, making up gas mixtures, equilibration, and analyses were those described in earlier papers by Black (1940) and Black et al. (1966a, b). All samples were analysed in the Department of Physiology, Dalhousie University. Liquid heparin (Connaught Laboratories) was used as the anticoagulant in the ratio of 1 ml of heparin to 10 ml blood. Pooled samples of blood were used in one-half of the experiments. Single samples of blood were used for carbon dioxide dissociation curves.

RESULTS AND DISCUSSION

Oxygen Capacities and Red Cell Volumes

The average oxygen capacities, red cell volumes, and the average oxygen capacities of 100 ml red blood cells for landlocked salmon acclimated to summer and winter conditions together with the data for the combined seasons are presented in Table I. The data of Black et al. (1966b) for Atlantic salmon are also included for comparison.

The average oxygen capacity, red cell volume, and the oxygen capacity of 100 ml red blood cells is much the same for summer and winter conditions. The averages for the combined seasons for oxygen capacity, red cell volume,

Table I. Combined summary of the oxygen capacities and red cell volumes for landlocked salmon corrected for heparin dilution and the oxygen capacities of 100 ml red blood cells taken during summer and winter. The data of Black et al. (1966b) on Atlantic salmon are included for comparison.

Season		Oxygen capacity (%)	Red cell volume (%)	Oxygen capacity of 100 ml cells (*vol* %)
Summer	Average ± SE	8.2±0.99	23.9±2.48	34.3±1.26
	Range	6.2–10.5	17.1–28.8	31.0–36.3
	n	4	4	4
Winter	Average ± SE	9.4±0.48	26.3±1.17	35.7±0.83
	Range	7.6–12.4	21.3–32.9	31.4–39.9
	n	10	10	10
Combined seasons	Average ± SE	9.1±0.45	25.6±1.11	35.3±0.70
	Range	6.2–12.4	17.1–32.9	31.0–39.9
	n	14	14	14
	Average ± SE	10.1±0.46	26.3±1.11	39.2±1.16
	Range	4.5–15.8	13.3–39.9	26.3–65.0
	n	35	34	34

and oxygen capacity of 100 ml red blood cells, are similar to the respective values reported by Black et al. (1966b) for Atlantic salmon.

Dissociation Curves

Oxygen dissociation curves were constructed from the blood of landlocked salmon at 5 (Fig. 1) and 25 C (Fig. 2). Samples of blood were taken from fish acclimated to both summer and winter conditions.

The water temperature, in which the fish acclimated to winter conditions lived, was approximately 5 C, whereas that for summer-acclimated fish was 20 C.

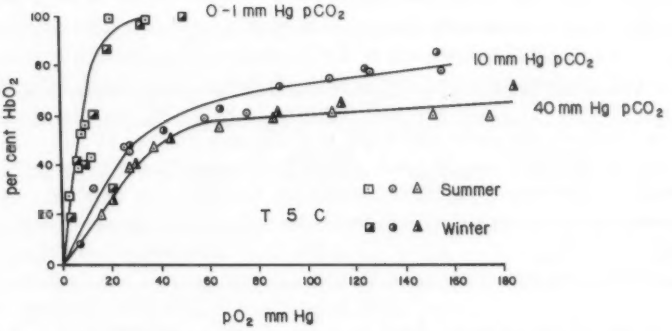

FIG. 1. Oxygen dissociation curves for landlocked salmon blood acclimated to summer and winter conditions at various tensions of carbon dioxide, equilibrated at 5 C.

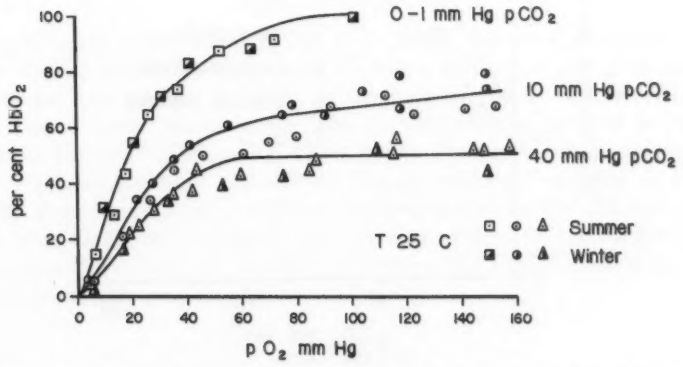

FIG. 2. Oxygen dissociation curves for landlocked salmon blood acclimated to summer and winter conditions at various tensions of carbon dioxide, equilibrated at 25 C.

Carbon dioxide dissociation curves for oxygenated and deoxygenated whole blood were also constructed at 5 C for fish acclimated to winter conditions (Table II, Fig. 3).

The effect of increased temperature upon the equilibrium between oxygen and hemoglobin is to shift the curves to the right (Table III, Fig. 1, 2), an effect first described for mammalian blood by Barcroft and King (1909). The quantitative influence is about the same for landlocked and Atlantic salmon (Table III), particularly when the oxygen dissociation curves are compared. (Fig. 1, 2 this paper; Black et al., 1966b, fig. 2, 5).

Examination of the curves for landlocked salmon at 25 C reveals that the single place where a difference is noted, i.e. $p\ CO_2$ of 40 mm Hg, the curve for the landlocked salmon is practically flat at the half-saturation value. At a tension of 45 mm Hg $p\ O_2$, the landlocked salmon blood is 43% saturated with oxygen.

TABLE II. Venous blood gases from one landlocked salmon acclimated to winter conditions at 5 C.

Experiment	Carbon dioxide (*vol %*)	Oxygen (*vol %*)	Oxygen capacity (*vol %*)	HbO_2 (%)	$p\ CO_2$ (*mm Hg*)	$p\ O_2$ (*mm Hg*)
Feb. 8, 1946	21.8	2.5	8.24	14.6	7	7

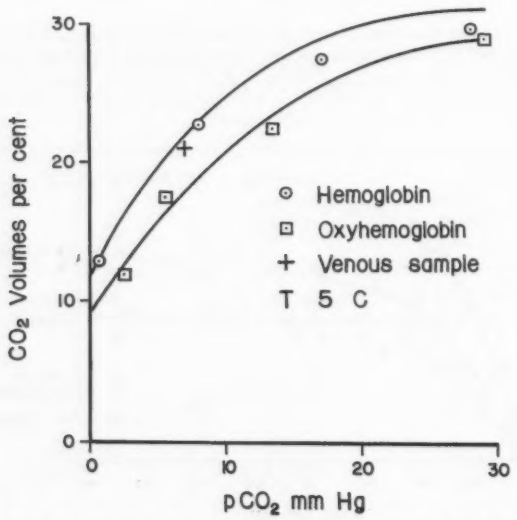

FIG. 3. Carbon dioxide dissociation curves for landlocked salmon blood acclimated to winter conditions and equilibrated at 5 C.

TABLE III. Tensions of unloading (tu = p O_2 for HbO_2 of 50%) for oxygen in landlocked and Atlantic salmon blood, equilibrated at two different temperatures and at three tensions of carbon dioxide.

Species	Temperature (C)	p CO_2 (0–1 mm Hg)	p CO_2 (10 mm Hg)	p CO_2 (40 mm Hg)
Landlocked salmon	5	8	31	43
Atlantic salmon	5	7.5	27	31
Landlocked salmon	25	19	37	80
Atlantic salmon	25	17	29	45

The Bohr effect, i.e. the influence of carbon dioxide in reducing the affinity of hemoglobin for oxygen, is pronounced in the landlocked salmon (Table III, Fig. 1, 2). The Bohr effect appears to be of the same magnitude in landlocked and Atlantic salmon (Black et al., 1966b; Benditt et al., 1941).

At 5 C (Fig. 1), an increase of 9 mm Hg p CO_2 increases the partial pressure of unloading at the half-saturation value, from 8 mm Hg p O_2 to 30 mm Hg p O_2. Thus, as was the case for the blood of Atlantic salmon, the Bohr effect serves to offset the disadvantage conferred by low environmental temperature for the landlocked salmon.

There does not appear to be any difference between oxygen dissociation data for summer- and winter-acclimated fish.

Carbon dioxide dissociation curves were constructed from the blood taken from a single fish (Table II, Fig. 3). The data are concordant with those reported for Atlantic salmon by Black et al. (1966, b). The tensions of carbon dioxide and oxygen for the single venous sample are 7 mm Hg each.

ACKNOWLEDGMENTS

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Erroneous Thiobarbituric Acid Values in Fish Tissues Caused by Their Normal Content of Free Iron[1,2]

By C. H. Castell and G. A. Boyce

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ABSTRACT

Erroneously high TBA values were obtained from liver, dark muscle, and certain other organs taken from seven species of sea fish. This error was eliminated by carrying out a portion of the test under anaerobic conditions or, more simply, by adding a mixture of EDTA and propyl gallate to the tissues immediately before the test is carried out. The error in the TBA values from the white muscle of lean fish and from whole cod fillets was found to be extremely small.

By adding measured amounts of iron to white muscle immediately before testing it was found that from 1 to 20 ppm Fe^{++} was sufficient to cause extensive errors in the TBA values. Schoenmakers and Tarladgis have recently reported that up to 5000 ppm Fe^{+++} can be added to methyl linolenate and several vegetable oils without changing the TBA values. It is suggested that this apparent contradiction is the result of the differences in the substrates used. Marine oils contain more highly unsaturated fatty acids and the tissues contain other biologically active compounds capable of reacting with the oxidizing lipids.

INTRODUCTION

In one form or another the thiobarbituric acid (TBA) reaction (Wilbur et al., 1949) is now extensively used as a measure of lipid oxidation in plant and animal tissues and in certain oils, fats, and fat-containing foods. It has the advantage over most other tests for oxidative rancidity in that it can be applied directly to the tissues without prior extraction. It has been accepted by some workers as a measure of "lipid peroxidation" (Barber, 1966; Bieri and Anderson, 1960; Gram and Fouts, 1966; Hunter et al., 1964; Meriwether and Mengel, 1966), because in some cases it has been shown that peroxidation and the formation of TBA-reactive compounds increase linearly (Täutel and Zimmerman, 1959; Dahle et al., 1962). The TBA-reactive substances (chiefly malonaldehyde: Sinnhuber et al., 1958; Kwon and Olcott, 1966) are carbonyls and are not peroxide in nature (Saslaw and Waravdekar, 1965; Saslaw et al.,* 1966). The malonaldehyde is a product of hydroperoxides which in turn are formed during the oxidation of certain unsaturated fatty acids (Meriwether and Mengel, 1966; Hoffman, 1962).

Because it is now so widely used, a knowledge of the limitations of this test becomes increasingly important. In relation to oxidative rancidity in fatty foods, Pohle et al. (1964) found that TBA values can be used as an index of quality in a given product or formulation, but the relative levels may vary from one product to another. For this reason the relationship between TBA

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[2]This is No. 8 in the series of papers dealing primarily with "Rancidity in lean fish muscle."

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values and rancidity would have to be established for each different food product before the TBA values could be used as an index of rancidity. Dahle et al. (1962) came to a similar conclusion by showing that the TBA colour developed from oxidized lipids will vary with the profile of polyunsaturated fatty acids existing in each sample.

Of more significance are the reports that the TBA test is subject to extensive error in tissues or other substrates to which inorganic iron has been added (Bernheim et al., 1948; Castell et al., 1966; McKnight and Hunter, 1965; Wills, 1964, 1965). In a more recent report Schoenmakers and Tarladgis (1966) question the seriousness of this error. Their results, using methyl linolenate, rape seed, soyabean, and groundnut oils showed that ferric ions, up to concentrations of 5000 ppm did not alter the TBA values, although concentrations of 10,000–100,000 ppm did. They rightly point out that such high concentrations of free iron can scarcely be expected in any plant or animal tissues. Castell et al. (1966), on the other hand, using fish muscle as the substrate, found that Fe^{++} and Fe^{+++} concentrations ranging between 5 and 20 ppm produced erroneously high values and that the error could be prevented either by replacing the oxygen in the distillation flask with nitrogen or by adding metal-binding agents such as EDTA or propyl gallate. McKnight and Hunter (1965) also reported that the error caused by the presence of inorganic iron could be prevented by carrying out the test under anaerobic conditions. It would appear that with some tissues at least, the presence of inorganic iron continues to accelerate oxidation of the lipids in the acidified tissues during the distillation or other heating that occurs while the test is being carried out. Castell et al. (1966) found that this error did not occur in the TBA values of cod muscle to which 50–1000 ppm of hemoglobin, myoglobin, hemin, or catalase were added immediately before testing.

Wills (1964) further suggests that the TBA reaction should be used with extreme precaution as a measure of lipid oxidation in certain tissues because of the error that might be caused as a result of the natural inorganic iron they contain. In this laboratory, abnormally high TBA values have been encountered in homogenates prepared from certain fish tissues, even when precautions had been taken to prevent the lipids from oxidizing prior to making the test, and where no inorganic iron had been added. This suggested an interference with the TBA test similar to that which occurs in fish muscle when traces of Fe^{++} or Fe^{+++} had been added, and prompted us to examine the possibility of error when this test is being used as a measure of lipid oxidation on tissues taken from various organs of marine fish.

PROCEDURE

The procedure used for determining the TBA values was that given by Tarladgis et al. (1960) in which the TBA-reactive compounds are separated from the tissues by distillation before being boiled with the TBA reagent. The chemicals used in preparing the reagent and for carrying out the test were

purified as recommended by Sinnhuber et al. (1958). The homogenates on which the tests were carried out were prepared by blending the tissue in a Waring blendor with three times its weight of distilled water. To determine whether any of the tissue lipids were being oxidized through the activity of naturally occurring metals, duplicate tests were made to which 0.5% each of EDTA and propyl gallate (PG) were added. It was found that a combination of both these reagents consistently gave better results than either one alone. As a further check, some duplicate tests were also made in which the air in the distillation flasks was replaced with nitrogen before the heat was applied. The difference in the TBA values obtained with and without these metal-binding compounds was taken as a measure of the error in the results. The term "TBA value" is used to indicate the optical density reading at 538 μm after reaction with the TBA reagent.

The tissues used were principally the white muscle and the dark lateral muscle of the fillet, and the liver. In some instances particular care was taken to prevent oxidation of the tissue lipids before the tests were carried out; in others the samples were purposely held for various periods at 0 C so as to allow some oxidation to take place before making the tests.

The tissues were taken from the following fish caught in the western Atlantic off the coast of Nova Scotia: Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus*), Atlantic cod (*Gadus morhua*), sea catfish (*Anarhicas lupus*), dogfish (*Squalus acanthias*), pollock (*Pollachius virens*), and thorny skate (*Raja scabrata*).

RESULTS

Fresh, Unoxidized Tissues

The lipids in some tissues rapidly oxidize when exposed to the air. For this reason, TBA values, with and without the addition of EDTA and PG, were obtained from the white muscle, dark muscle, and liver of two cod immediately after they had been killed:

Tissue	TBA Values with EDTA and PG	Controls	Difference
White muscle	0.020	0.023	0.003
White muscle	0.023	0.024	0.001
Dark muscle	0.035	0.063	0.028
Dark muscle	0.058	0.085	0.027
Liver	0.046	0.265	0.219
Liver	0.046	0.178	0.132

Because of the difficulty of removing the dark muscle and separating it from adhering portions of white muscle, the lipids had a greater chance of being

oxidized prior to the test than those in the white muscle or liver. It can be seen that without the addition of the metal-binding reagents there was little or no error in the values for the white muscle; there was an obvious increase in the values for the dark muscle and a relatively large error in the values for the liver.

A series of ten replicate tests was also made on the white muscle from one recently killed cod with the following results:

	TBA Values with EDTA and PG	Controls
Mean	0.0238	0.0247
Standard deviation	0.00313	0.00305
Standard error	0.0007	0.0007

These results show that by the use of the metal-binding compounds in the tissues it is possible to get low TBA values from all three tissues when they are in a very fresh condition; and that any error resulting from iron in the white muscle is exceedingly small.

Partially Oxidized Tissues

TBA values were also obtained from a large number of samples that had undergone various degrees of lipid oxidation before the tests were made. This was accomplished by leaving the whole organs or the blended tissues at 0 C for different periods of time after they had been cut from the fish. The results for the liver, dark and light muscle from seven species of fish are shown in Fig. 1. In preparing the figure, the results from each species have been arranged in order of decreasing rancidity as indicated by the TBA values where EDTA and PG were used. It should also be pointed out that the values shown for the livers do not always correspond to the values for the dark or light muscle in the columns immediately below. Again it can be seen that the error, as shown by the values obtained when the metal-binding compounds had been omitted, was greatest with the liver, next with the dark muscle, and least with the white muscle. There were exceptions to this in the case of the herring and mackerel. In contrast to the "lean" fish these "fatty" fish showed the greatest error in the TBA values from the dark muscle, and a relatively small error in the results from the liver.

A cod liver was removed from a large fish and stored for 18 hr at 0 C in a covered, but not air-tight glass container. It was then blended as usual with three times its weight of water and the blend put back at 0 C. One TBA test was made immediately and seven others at 20-min intervals. The results are shown in Fig. 2. The lipids in the liver had been considerably oxidized by standing for the 18 hr before the first test was made, and they continued to oxidize while the successive TBA tests were being carried out. The difference in the

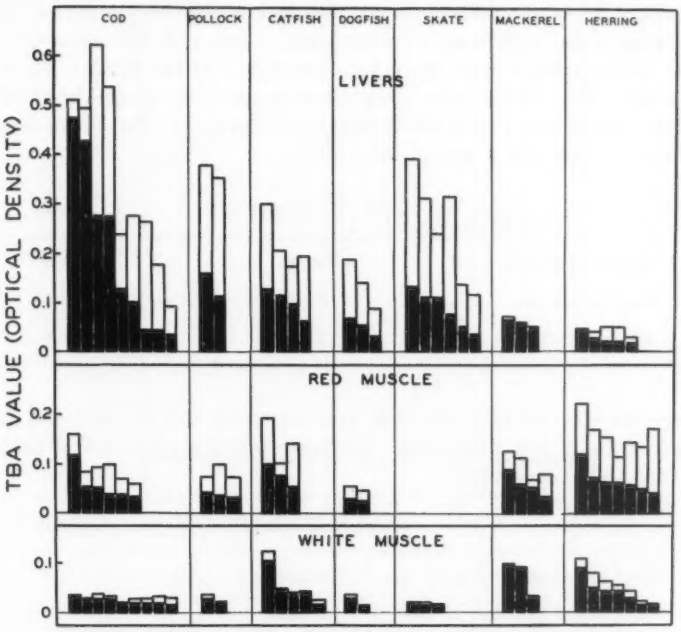

FIG. 1. TBA values of liver, dark muscle, and light muscle of seven species of fish. The black columns give the values obtained when EDTA and PG were added to the tissues before the tests were made. The white columns indicate the excess in the TBA values that occurred when these metal-binding agents were omitted (the samples have been arbitrarily arranged in order of descending rancidity).

heights of these two curves indicates the extent of error caused by the uncontrolled oxidation taking place while the distillation was being carried out.

In some tests the air in the tissues in the distillation flasks was withdrawn and replaced with nitrogen. This had the same effect as the EDTA and PG on liver and dark muscle in that it prevented the development of falsely high TBA values.

MEASURING RANCIDITY IN WHOLE COD FILLETS

The proportion of white and dark muscle differs in different species of fish; it also differs in different areas of the fillet. In haddock (which is quite similar to cod), the dark muscle accounts for approximately 8% of the fillet in the tail section and closer to 1% in the upper, thicker portions of the fillet (Fraser et al., 1961). In contrast to this, these same workers found that the dark muscle accounted for approximately 14% of the total weight in mackerel fillets. As the dark muscle is subject to a greater error in TBA value than white muscle, the question arises as to the extent of error caused by the dark

muscle when the whole fillet is tested. To answer this in relation to cod fillets the following experiments were carried out. Three cod fish were filleted and the right and left hand fillets were kept separate. On the three fillets from the left side the TBA values were determined separately on the light and dark muscle; on the other set the TBA values were made on the whole fillets. The mean values for the three sets of tests were:

	With EDTA and PG	Controls
White muscle only	0.021	0.024
Dark muscle only	0.035	0.068
Whole fillets	0.022	0.026

In the second test, 80–90-g portions were cut from the tail end and from the head end of three cod fillets that weighed approximately 400 g each. Each portion was tested separately:

	With EDTA and PG	Controls
Head end of fillet No. 1	0.026	0.026
Head end of fillet No. 2	0.022	0.016
Head end of fillet No. 3	0.023	0.024
Tail end of fillet No. 1	0.019	0.025
Tail end of fillet No. 2	0.004	0.026
Tail end of fillet No. 3	0.023	0.029

From these results, by inspection, it can be seen that the dark muscle in cod fillets is likely to produce only a very insignificant error in the values obtained from the whole fillet. Further testing along this line was not continued, but one would suppose from the results already obtained, that the dark muscle in herring and mackerel would contribute more to the error in the TBA values for the whole fillets than was the case in the cod.

Effect of Added Blood

In a previous paper (Castell et al., 1966), it was shown that the addition of hemoglobin immediately before making the TBA test did not increase the values over those from the fish without this addition. In this present work a number of tests were made in which whole blood, taken from the heart of recently killed fish was added to blended white muscle. With amounts up to one part of blood to nine parts of muscle, by weight, there was no evidence to indicate that the blood catalyzed lipid oxidation in the acidified tissue during distillation.

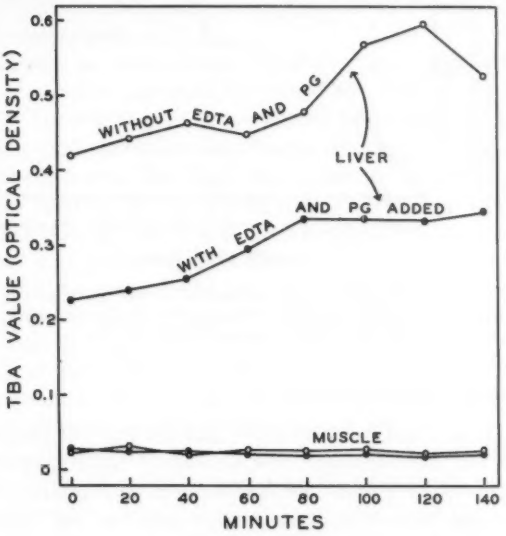

FIG. 2. Effect of adding EDTA and PG on TBA values obtained from partially oxidized cod liver and fresh cod white muscle. The tissues were blended and divided into eight portions which were held at 0 C. Every 20 min samples were withdrawn from the cold storage and tested. The dark circles represent the values with the metal-binding agents added and the white circles the values when they were left out.

MINIMUM CONCENTRATIONS OF Fe^{++} REQUIRED TO PRODUCE ERRONEOUS VALUES DURING THE TBA TEST ON COD MUSCLE

Fe^{++} was used in these experiments instead of Fe^{+++} because it had been found in a very large number of tests (still unpublished) that ferrous ions were much more effective catalysts of lipid oxidation in cod muscle than ferric ions. Smith and Dunkley (1962) have similarly found that Fe^{++} was more effective than Fe^{+++} in initiating oxidation of linoleate.

Fe^{++}, as ferrous sulphate, in amounts to give concentrations ranging from 0.01 to 20 ppm, was added to blended cod muscle immediately before it was tested for TBA values. In each case the tests were made with and without the addition of EDTA and PG. The results of one experiment (Fig. 3) show that for that particular sample of cod muscle, less than 1 ppm was sufficient to produce erroneous results. However, in other tests as much as 5 ppm had no effect. In every instance, however, 20 ppm Fe^{++} produced a large error. From these results it would appear that the minimum concentration of Fe^{++} necessary to cause erroneously high TBA values with cod muscle varies slightly with different fish or fish having different treatment prior to making the test.

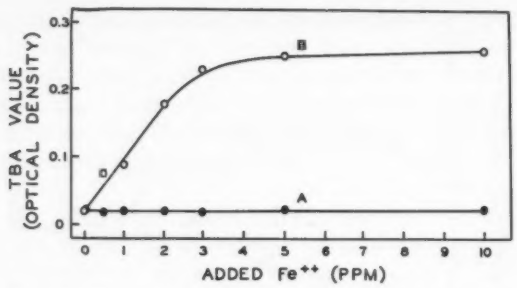

FIG. 3. Effect of concentrations of added Fe^{++} on the error in the TBA values of fresh cod muscle; (A) with, and (B) without added EDTA and PG.

EFFECT OF OTHER HEAVY METALS

It has been shown that Fe^{+++} or Fe^{++}, but not Cu^{++} or hematin compounds, when added to the homogenate, will accelerate lipid oxidation during the distillation. Other heavy metals are also known to accelerate lipid oxidation. It seemed of interest, therefore, to determine whether any other metals would similarly catalyse oxidation during the distillation of the acidified fish muscle. To do this, sulphates or chlorides of cobalt, nickel, manganese, zinc, cadmium, chromium, cerium, copper, and iron were added to homogenized cod muscle to give concentrations of 20 ppm of the metal ions and then immediately tested for TBA values without the addition of EDTA and PG. The resulting TBA values were as follows: Control 0.020, Co^{++} 0.020, Ni^{++} 0.025, Mn^{++} 0.020, Zn^{++} 0.030, Ca^{++} 0.025, Cr^{+++} 0.030, Ce^{++} 0.030, Cu^{++} 0.040, Fe^{++} 0.310, and Fe^{+++} 0.570. This strongly suggests that iron, rather than some other heavy metal, is the agent involved.

EFFECT OF HEATING TISSUES PRIOR TO TESTING

It was observed that boiling or autoclaving tissue from various organs of the fish prior to testing increased the TBA values, especially if this was carried out in a closed vessel so that the volatiles were not driven off. It was also observed that when tests were carried out on these previously heated tissues, with and without the metal-binding compounds, the liver and red muscle still produced erroneously high TBA values.

TESTS ON ORGANS OTHER THAN LIVER AND MUSCLE

A few tests were also carried out on heart, spleen, gonads, roe, intestines, and pyloric caecum from several different species of sea fish. None of these tissues produced errors as great as those observed with the liver from lean fish, but the heart, spleen, mature ovaries, and roe consistently gave higher TBA values when the EDTA and PG were omitted. Other organs, such as the intestine, pyloric caecum, and testicles gave approximately the same values

with and without the metal-binding compounds. In this way they were quite similar to the white muscle.

For the purpose of comparison, tests were also made on beef and pork livers. Instead of the red pigment, typical of the TBA reaction with oxidized fish lipids, these mammalian livers produced bright orange or yellow-orange colours whose optical densities were more effectively measured at a wave length of 440 μm. (It was also observed, incidentally, that pollock heart and testicles, and the liver of the fatty fish, such as herring or mackerel, produced larger amounts of orange than red pigments when the distillates reacted with the TBA reagent.) It was presumed that the beef and pork livers may have had an insufficient amount of the highly unsaturated fatty acids which are the necessary precursors of the malonaldehyde that produces the red colour with the TBA reagent. For this reason the test with the beef liver was repeated, except that 1% of an oil extracted from the bellyflap of mackerel was also added. In this case the TBA values without the EDTA and PG was 0.250; when the metal-binding reagents were added the value was 0.205. These results are not very conclusive except to indicate that the TBA reaction on the lipids from the mammalian livers was different from that obtained with most of the fish livers.

DISCUSSION

These results, showing the effect of iron on the TBA values of tissues from marine fish, are widely different from those recorded by Schoenmakers and Tarladgis (1966) for methyl linolenate and several vegetable oils. They found that up to 5000 ppm Fe^{++} could be added immediately before making the test without affecting the TBA values. We have found that not only 1–20 ppm added Fe^{++}, but also the naturally occurring metal in some fish tissues, is sufficient to produce highly erroneous TBA values. The greatest error resulting from the natural iron content of the tissues was encountered in the liver of lean fish, such as cod, pollock, dogfish, or skate. Wills (1966) has pointed out that peroxide formation in animal tissues is catalyzed both by haemoproteins and by inorganic iron, or ferritin in the presence of ascorbic acid. All tissues tested contained both types of catalysts but liver, kidney, and heart contained proportionally greater amounts of the non-haem iron. Errors in TBA values were also found with the dark lateral muscle of all the fish tested and with heart spleen and some of the other internal organs. The white muscle of the lean fish, on the other hand, gave approximately the same TBA values with and without the added metal-binding compounds. This is not unexpected. Compared to other tissues the white muscle is relatively low in total iron and therefore still lower in non-haem iron. Nilson and Coulson (1939) reported that cod muscle (presumably both white and dark muscle) contained approximately 5.18 ppm iron. Dambergs (personal communication) made many iron determinations on the white muscle of cod and found they ranged between 1.0 and 5.5 ppm, averaging approximately 4 ppm, but no values were obtained for the free iron content. Dark muscle of fish is known to contain much more

iron than white muscle (Parks and Rose, 1933; Nanicki, 1934) and is comparable in this way to the flesh of beef and pork (Borgstrom, 1962).

The apparent contradictions in the results of Schoenmaker and Tarladgis and those recorded here are probably caused by the difference in the substrates used. The fish tissue lipids not only have a higher content of polyunsaturated fatty acids, but they also contain many biologically active compounds in the cells that are capable of reacting with the oxidizing lipids. It is known, for example, that ascorbic acid has an accelerating effect on lipid oxidation (Deutsch et al., 1941; Ottolenghi, 1959; Thiele and Huff, 1964; and many others). More specifically Elliott and Libet (1944) and Barber (1966) have shown that ascorbic has a marked effect on the oxidation of phospholipids catalyzed by iron.

Although the procedure used here has been the distillation method of Tarladgis et al. (1960), it would be expected that similar errors resulting from the presence of free iron would be encountered in any other procedure in which acidified tissues are subjected to heat in order to separate the TBA-reactive compounds. With some fish tissues at least, the warning of Wills (1964) that the results of the TBA test as a measure of lipid oxidation in tissues must sometimes be taken with extreme caution, is amply justified.

It has been shown that with certain very freshly excised fish tissues (i.e. liver and dark muscle) an initial low TBA value cannot be obtained unless the test is carried out under anaerobic conditions, or when reagents have been added to the tissue that will immobilize the iron they contain. Although this precaution is not necessary for all tissues, such as the white muscle of most lean fish, it certainly should be taken into consideration where this characteristic of the tissue has not been determined. Although most fillets are composed of both light and dark muscle, it has been shown that with cod the error caused by a relatively small proportion of red muscle is so small that for all practical purposes it can be neglected.

A simple modification to the TBA test that will eliminate this error caused by the iron normally in the tissues (or added inorganic iron), is to add a mixture of EDTA and propyl gallate to the tissues before the test is carried out. It can be added directly to tissues that are being blended or to the tissues in the distillation flasks before any heat is applied. We have found a 1 to 1 mixture of EDTA and PG, in an amount equal to 1% of the weight of the tissue being tested, to be satisfactory except where very high concentrations of free iron have been added (Castell et al., 1966).

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Comparative Electropherograms of *Coregonus clupeaformis*, *Salvelinus namaycush*, *S. alpinus*, *S. malma*, and *S. fontinalis* from the Family Salmonidae[1]

By H. Tsuyuki, J. F. Uthe, E. Roberts, and L. W. Clarke

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver 8, B.C.

ABSTRACT

Zone electropherograms of muscle myogens and blood proteins of members of the family Salmonidae revealed in addition to species specificity, marked similarities at the genus and family levels. In genera investigated, other than the *Oncorhynchus*, intraspecies polymorphisms were found. Two general hemoglobin patterns were uncovered from specimens of *Coregonus clupeaformis* from Great Slave Lake while their muscle myogen patterns were specifically uniform. *Salvelinus namaycush* from Lake Superior and Cayuga Lake were readily distinguishable by differences in their multiple hemoglobin patterns as well as by plasma proteins as revealed by polyacrylamide disc electrophoresis. Muscle myogen electropherograms of *S. namaycush*, *S. alpinus*, and *S. malma* showed striking similarities, the latter two being almost superimposable while the patterns of *Salvelinus fontinalis* were more closely allied to that of the genus *Salmo*. The value of muscle myogens in phylogenetic studies and in intraspecies protein variations as diagnostic characters in stock analyses is discussed.

INTRODUCTION

In the family Salmonidae, members of the genus *Oncorhynchus* have been subjected to extensive zone electrophoretic investigation for both species specificity and geographical constancy in muscle myogen and blood protein patterns (Tsuyuki et al., 1965a, b). The development of standardized zone electrophoretic techniques for subsequent studies has been worked out largely with this genus which coincidentally was also characterized by its biochemical uniformity. This facilitated the formulation of the bases for species specificity and for the relationship of muscle myogen electropherograms at the generic and family levels (Tsuyuki and Roberts, 1966). Other than ontogenetic (Vanstone et al., 1964) changes and the seldom occurring odd patterns, the hemoglobin electropherograms of the genus *Oncorhynchus* are relatively uniform. Adult *Salmo salar* is also noted for the absence of variations in hemoglobin patterns (Koch et al., 1964) once ontogenetic changes have taken place. In contrast, genetically interpretable hemoglobin polymorphisms have been observed in whiting, *Gadus merlangus*, and the Atlantic cod, *Gadus morhua* (Sick, 1961; Frydenberg et al., 1965), providing a useful biochemical basis

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for racial or subpopulation studies, which in the past have been approached largely through an analysis of meristic and morphometric characters. Though mainly on theoretical grounds, biochemical traits have been variously considered to be relatively uninfluenced by environmental factors while nearly every meristic series of characters subjected to controlled experimental conditions have been known to vary (Lindsey, 1961). Polymorphisms of this type have also been found in the muscle myogens of *Anoplopoma fimbria* (Tsuyuki et al., 1965a) and *Stizostedion vitreum* (Uthe et al., 1966) indicating that interspecies genetic differences are not necessarily confined to a single group of proteins nor for that matter in every species examined. A number of protein groups should therefore be analysed.

In this study electrophoretic analyses of muscle myogens and blood proteins are extended to other members of the family Salmonidae, specifically, lake whitefish (*C. clupeaformis*), lake trout (*Salvelinus namaycush*), arctic char (*S. alpinus*), brook trout (*S. fontinalis*), and Dolly Varden (*S. malma*). Where possible, each species is examined for intraspecies variations, within as well as between various geographical areas, and their significance discussed.

MATERIALS AND METHODS

Samples of *C. clupeaformis* were obtained from Lake Huron, Cayuga Lake, Great Slave Lake, and Great Bear Lake; *S. namaycush* from Lake Superior, Cayuga Lake, and Great Bear Lake; *S. fontinalis* from two lakes in New York, Honnedaga, and Horn.

The muscle extracts and blood proteins were prepared and separated by starch gel electrophoresis as previously described (Tsuyuki et al., 1965a; Vanstone et al., 1964). Disc electrophoresis was carried out with the Model 12 Canalco instrument as before (Tsuyuki and Roberts, 1965).

RESULTS

Lake Whitefish

The muscle myogens from a total of 48 specimens, 12 from Lake Huron, 32 from Great Slave Lake, 2 from Cayuga Lake, and single samples from Great Bear Lake and Lake Superior were subjected to starch gel electrophoresis. For an exact comparison specimens from four areas were analyzed simultaneously (Fig. 1A). The patterns were essentially identical, the minor quantitative variations being within the limits of those found in the 11 randomly selected specimens of both sexes from Great Slave Lake shown for this purpose in Fig. 1B. Sexual dimorphism was not evident in the 15 female and 17 male specimens analyzed from Great Slave Lake including the patterns shown in Fig. 1. The single lake trout pattern included in Fig. 1A points out the many common protein zones shared by the two species belonging to the same family.

In contrast to the myogens, plasma protein patterns varied in as many individuals as examined and is shown in its entirety to bring out the full range

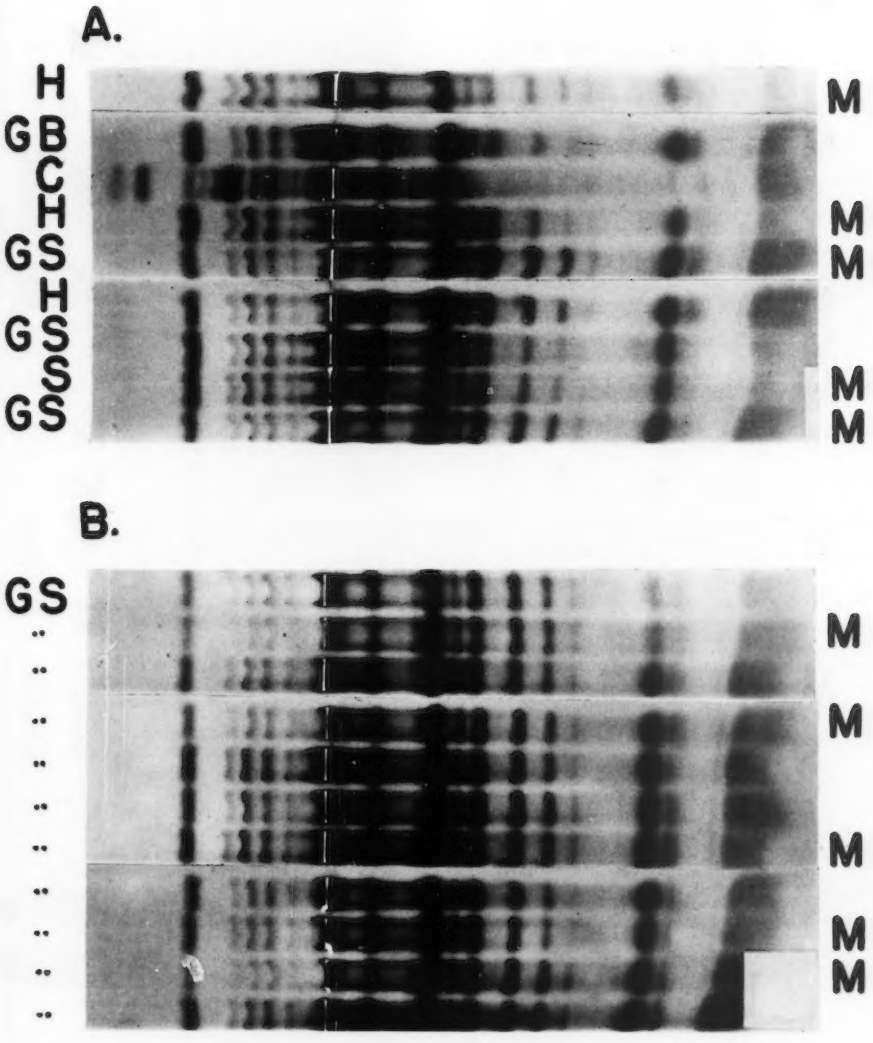

FIG. 1. A. Starch gel electropherograms of muscle myogens of *Coregonus clupeaformis* from Great Bear Lake (GB), North channel of Lake Huron (H), Great Slave Lake (GS), and Lake Superior (S). One sample of *Salvelinus namaycush* from Lake Cayuga (C) is included for comparison. Male specimens are designated M. The remainder are females. The anode is to the right in this and subsequent figures. B. A simultaneous starch gel electropherogram of muscle myogens of *Coregonus clupeaformis* from Great Slave Lake (GS).

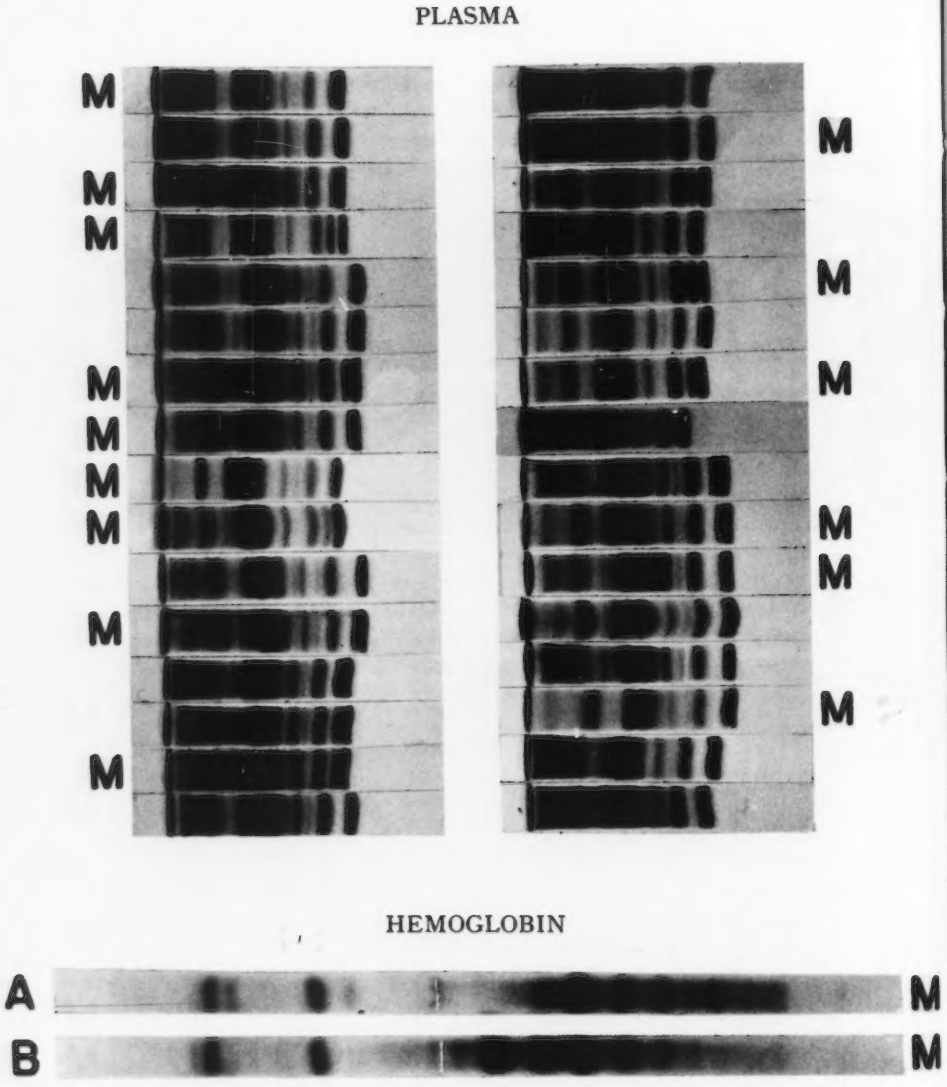

FIG. 2. Polyacrylamide disc electropherograms of plasma proteins from 32 specimens of *Coregonus clupeaformis* from Great Slave Lake. Representatives of two types (A and B) of hemoglobin patterns from the same species from this area are also shown. Male specimens are designated M while the remainder are females.

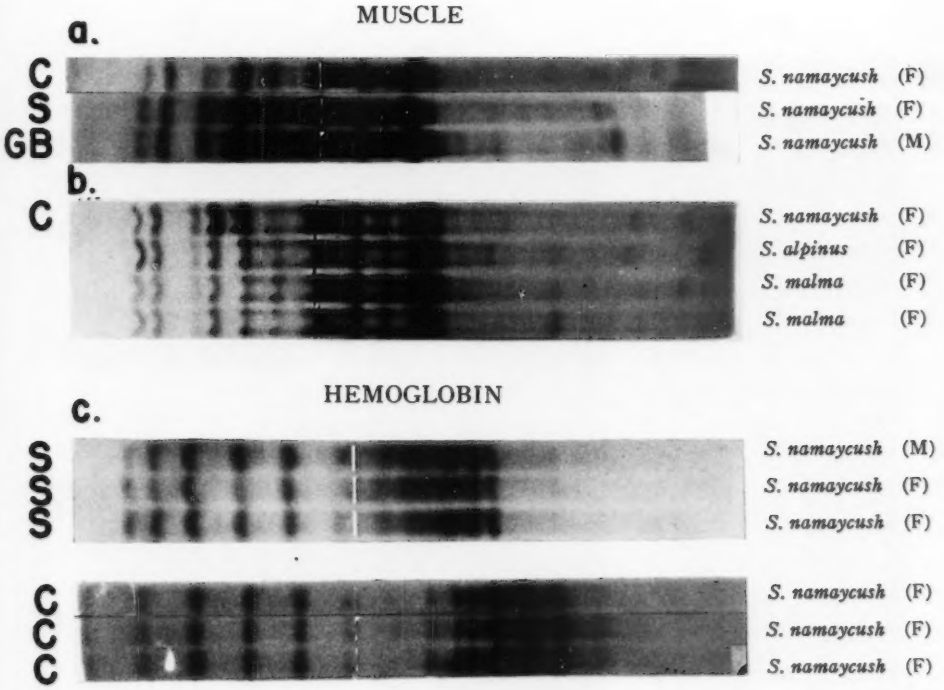

FIG. 3. (a) Starch gel electropherograms of muscle myogens of *S. namaycush* from Lake Cayuga (C), Lake Superior (S) and Great Bear Lake (GB). (b) A direct comparison of starch gel electropherograms of muscle myogens of *S. namaycush*, *S. alpinus*, and *S. malma*. (c) Comparative starch gel electropherograms of the multiple hemoglobins of *S. namaycush* from Lake Superior (S) and Lake Cayuga (C). Letters M and F refer, respectively, to male and female specimens.

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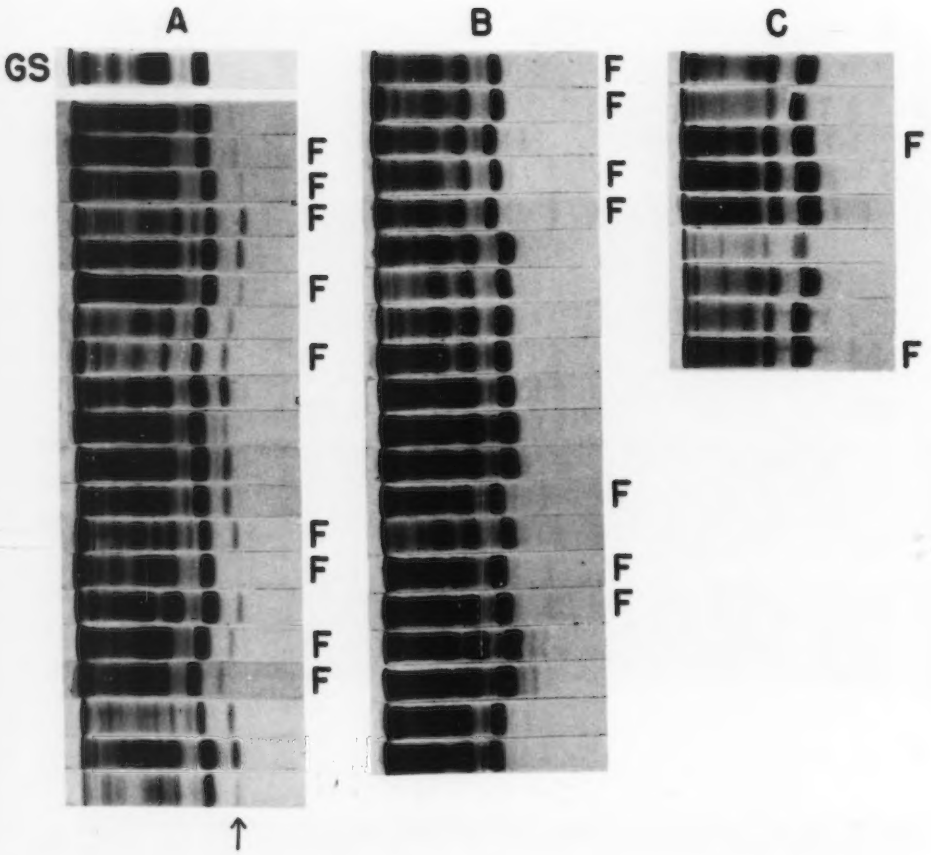
A
B
C
GS
F
F
F

MUSCLE

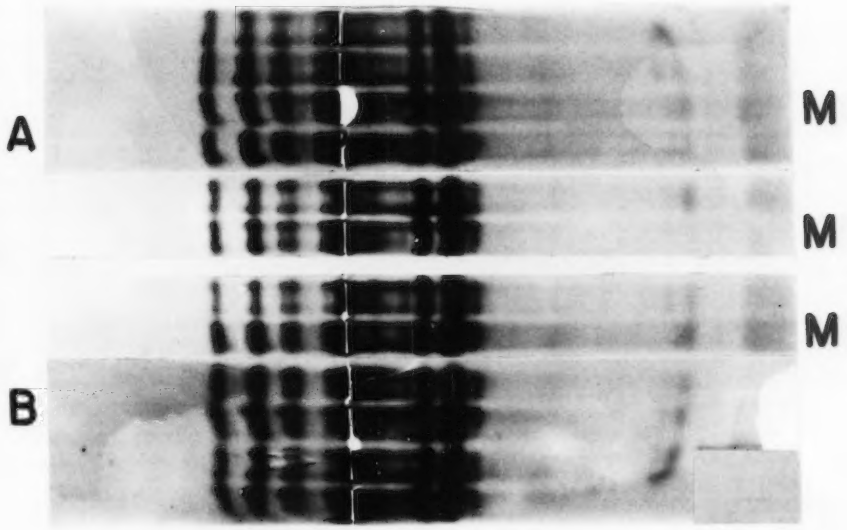

FIG. 5. Comparative starch gel electropherograms of the muscle myogens of *S. fontinalis* from Honnedaga Lake (A) and Horn Lake (B). Male specimens are designated by the letter M, while the females are unlettered.

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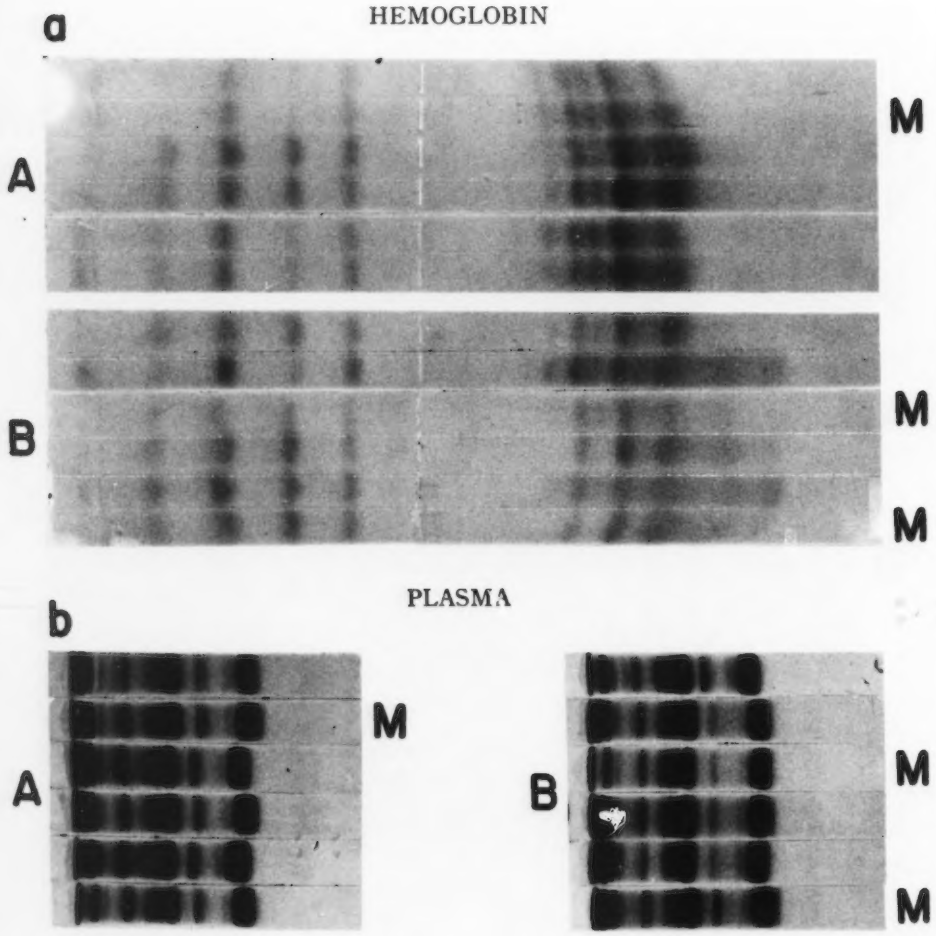

FIG. 6. (a) Comparative starch gel electropherograms of the multiple hemoglobins of *S. fontinalis* from Horn Lake (A) and Honnedaga Lake (B). (b) Comparative polyacrylamide disc electropherograms of the plasma proteins of *S. fontinalis* from Horn Lake (A) and Honnedaga Lake (B). Male specimens are designated by the letter M while the females are unlettered.

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of variations that could be expected (Fig. 2). Systematic variations which might enable segregation of Great Slave Lake whitefish in subgroupings were not evident from the disc electrophoretic patterns of total plasma proteins.

Of 38 hemoglobin samples analyzed, however, 22 displayed type A and 16 type B patterns. Since all samples analyzed fell into the two categories, representative patterns of each are shown in Fig. 2. Type A is somewhat similar to that of the rainbow trout from previous studies. Considerable care must be exercised in interpreting hemoglobin patterns for it is known that storage produces changes in patterns. In these specimens the length of time involved between the preparation of hemoglobin samples at Great Slave Lake and shipment to Vancouver, B.C., for electrophoretic analyses, could have caused some chemical changes in the hemoglobin molecules.

Lake Trout and Arctic Char

The widely distributed lake trout (Lindsey, 1964) has not developed distinguishable morphological differences during its long isolation in geographically separated lakes (Rounsefell, 1962). However, the biochemical uniformity of this species from different areas has not yet been investigated. Representative adult specimens of lake trout from Lake Cayuga, Lake Superior, and Great Bear Lake run simultaneously, displayed the same basic myogen patterns (Fig. 3a). Forty other individuals from the three localities likewise showed similar patterns with minor variations. The specificity of the pattern in relation to lake whitefish is evident from Fig. 1 and to alpine and speckled trouts in a previous study (Tsuyuki and Roberts, 1965). The greater intensity of the most anodic zone in the previous study may have resulted from the juvenile captive fish employed.

The muscle myogen patterns of *S. namaycush*, *S. alpinus*, and *S. malma* are compared in Fig. 3b. These samples were analyzed after approximately 8 months frozen storage, a factor that does introduce some changes as evident by comparison with the patterns of fresh samples of *S. namaycush* in Fig. 3a. *Salvelinus fontinalis* is not included in these electropherograms for a direct comparison as samples were not available. Of the three species of the genus *Salvelinus* shown here, *S. namaycush*, though very similar, is specifically different from *S. alpinus* and *S. malma*, and the latter two have patterns which are practically superimposable except for a couple of minor zones with extreme anodic mobility, indicating very close genetic relationship.

The hemoglobins of lake trout from Lake Superior and Cayuga Lake are compared (Fig. 3c). The three patterns of each type are representatives from 20 Lake Superior and 22 Cayuga Lake specimens. Readily distinguishable mobility differences are found in both cathodal and anodal zones from these specimens from the two lakes.

The disc electrophoretic plasma protein patterns of one Great Slave Lake, 20 (7 female, 13 male) Cayuga Lake (Fig. 4A), and 20 (9 female, 11 male) Lake Superior (Fig. 4B) specimens of *S. namaycush*, point out the range of variations obtained in total plasma protein analyses of this species. No

sexual dimorphism is evident in the specimens from each lake system. There is, however, a marked difference in the presence of one and in some cases two zones corresponding to prealbumins (shown by arrow in Fig. 4) in the Cayuga Lake samples. Only one of 20 lacked this zone while only a few of 20 possessed even a trace of a double or single protein zone in the Lake Superior samples. In this respect the lone Great Slave Lake (Fig. 4A) specimen is similar to the Lake Superior patterns. The plasma protein patterns of two female and seven male juvenile *S. alpinus* (Fig. 4C) displayed a peculiar double zoning in the albumin region.

Brook Trout

The muscle myogen patterns of two wild strains of *S. fontinalis* from two lakes in New York State, Honnedaga (Fig. 5A) and Horn (Fig. 5B), are virtually indistinguishable. These patterns agree reasonably well with those in two other studies (Tsuyuki and Roberts, 1963, 1965). From these and other investigations the myogen patterns of *S. fontinalis* are specifically different from 16 salmonid species examined.

The hemoglobin patterns of the two strains of *S. fontinalis* (Fig. 6a) appear to fall into two groups. Four of the Honnedaga Lake variety (Fig. 6aB) show one type, and two the other, while all six from Horn Lake (Fig. 6aA) are uniform. The differences are confined only to the anode side, the Horn Lake variety exhibiting two additional anodic zones (zone 1 and 3 from the origin). The fastest anodic zones are not considered at this time as these often appear after a period of storage though not at the expense of zones 1, 2, and 3.

The disc electrophoretic plasma protein patterns of the two groups were relatively uniform (Fig. 6b) with the exception of variations found in the three zones adjacent to the origin. Two out of six specimens from both lakes showed one missing zone in this region.

DISCUSSION

In the family Salmonidae the blood and tissue proteins of the genus *Oncorhynchus* have been subjected the most extensively to electrophoretic analyses (Tsuyuki et al., 1965a, b; Tsuyuki and Roberts, 1966). In comparison to other species this genus is characterized by the relative absence of intraspecies protein polymorphisms as revealed by zone electrophoretic analyses of their muscle myogens, blood hemoglobins, and to a lesser extent, plasma proteins. Similar observations were reported for the multiple hemoglobins of *Salmo salar* (Koch et al., 1964). However, it does not necessarily follow that other tissue proteins likewise exhibit the same degree of biochemical uniformity.

Other members of the family examined in this investigation, however, revealed varying degrees of polymorphism in certain protein groups. The electrophoretic patterns of muscle myogens of *C. clupeaformis*, *S. namaycush*,

S. alpinus, *S. malma*, and *S. fontinalis* are species specific, free from intraspecies variations other than minor quantitative differences, and reflect intrafamily relationships. These family resemblances are manifested in protein zones of similar mobilities and indicate the presence of protein biosynthetic systems under similar genetic control, although the similarity in the mobilities of proteins does not necessarily indicate identity in the primary structures of proteins. At the generic level pattern resemblances become stronger as shown by the comparative electropherograms of *S. namaycush*, *S. alpinus*, and *S. malma*, the latter two being practically superimposable except for variations in the extreme anodic minor zones. Muscle myogen patterns of this order of similarity are expected at the subspecies level. In fact, some investigators (Dymond, 1936, 1947; Carl and Clemens, 1953; Lindsey, 1956) considered *S. malma* a part of the *S. alpinus* complex and referred to it by the subspecific name of *S. alpinus malma*, while others (Delacy and Morton, 1943; Morton and Miller, 1954; McPhail, 1961) reported these to be two separate species. The muscle myogen pattern of *S. fontinalis* is phylogenetically more distantly related to the other members of the genus *Salvelinus* and shows increasingly more of the mobility characteristics common to *Salmo gairdneri* to which it is compared in previous publications (Tsuyuki and Roberts, 1963, 1965). A direct comparison of *S. gairdneri*, *S. fontinalis*, and *S. malma* in those reports emphasizes this point. It is interesting that in a taxonomic study of the eastern North American chars, Vladykov (1954) has placed *S. fontinalis* in a subgenus, *Baione*.

Of the fish protein groups examined in these studies the muscle myogens most clearly reflect genetic similarities and their electropherograms follow closely the phylogenetic relationships established by classical approaches. Those findings are in agreement with previous observations in the genus *Oncorhynchus* (Tsuyuki and Roberts, 1966) and in the genus *Sebastodes* (Tsuyuki et al., 1965).

Polymorphisms in multiple hemoglobins appear to be more common than in muscle myogens in fishes examined so far. Hemoglobin polymorphisms have, for instance, been investigated extensively in the Atlantic cod, *Gadus morhua*, and the frequency of occurrence of the gene responsible for the formation of one type of hemoglobin correlated with geographic area (Sick, 1961; Frydenberg et al., 1965). Protein polymorphisms, therefore, promise to have wide application as chemical markers in this type of stock analyses. In the family Salmonidae similar hemoglobin polymorphisms were found in *C. clupeaformis*, *S. namaycush*, and *S. fontinalis* while an analysis of large numbers of the genus *Oncorhynchus* revealed a marked degree of uniformity (Tsuyuki et al., 1965b). Usually, there is no clear-cut indication of the possible existence of intraspecies protein polymorphisms. The hemoglobins are no exception and necessitate a survey of large numbers of specimens of various species. The two general types of hemoglobin patterns in *C. clupeaformis* indicate the existence of two subgroups of this species in Great Slave Lake. Caution, however, should be exercised in the interpretation of hemoglobin

patterns as we have experienced (unpublished data) the formation of spurious zones upon storage. Methemoglobin is believed to be formed upon storage (Yamanaka et al., 1965). Some uncertainty appears to exist in the classification of whitefishes (Koelz, 1929; Svardson, 1957; Lindsey, 1962) and the seven subspecies of lake whitefishes described (Koelz, 1930) may in part reflect the blood hemoglobin polymorphism of this species.

The observed variability of total plasma protein electropherograms of *C. clupeaformis* populations from Great Slave Lake has been indicated by other studies (Booke, MS, 1962). The usefulness of such patterns in biochemical systematics of fishes is limited by physiological, serological, sexual, and other factors affecting the concentration of the individual proteins normally present, as well as the formation of new proteins (Booke, 1964). In *Petromyzon marinus* (Uthe et al., 1966), for example, parasitic stage of the animal conferred vast changes. In the absence of any systematic variations, it is difficult, therefore, to give meaningful interpretation to the disc electropherograms of *C. clupeaformis* except to point out the full range of variations to be expected. No definite relationship could be established with the two general hemoglobin patterns. This finding, however, does not rule out the undoubted value of genetically linked polymorphisms in classes of proteins within the total serum proteins. Serum transferrins (in literature bibliography by Wye, 1964) and albumins (Quinteros et al., 1964; Stormont and Suzuki, 1963) for example, have been of considerable value in the stock analysis of warm-blooded animals. Recently, serum transferrin polymorphisms were found in some gadoid fishes (Moller and Naevdal, 1966). In *S. namaycush* consistent differences were revealed in the blood proteins of specimens from two lakes. Cayuga Lake specimens differed from those of Lake Superior in possessing zones corresponding to the pre-albumins. It may be well to point out again that the hemoglobin patterns of this species from the two lakes differed considerably. Definite genetic differences, manifested in the blood proteins, therefore exist in *S. namaycush* from the two lakes. The lake trout of Cayuga Lake in New York and the sea lampreys have coexisted though the latter species are a slightly smaller landlocked race (Wigley, 1959).

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Morphology of the Digestive Tract of the Cod, *Gadus morhua*[1]

By C. Bishop and P. H. Odense

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ABSTRACT

The morphology of the digestive tract of the cod (*Gadus morhua*) was studied from dissections and histological sections.

The stomach of the cod is between the siphonal and caecal types described by Grasse; and has well-developed glands compared to other teleosts. Glands are also present in the numerous pyloric caeca and intestine, and it appears that the constant replacement of the epithelial cells occurs from these. "Pear-shaped" cells which could be enzyme-producing are present in both the epithelium and glands of the ileum and pyloric caeca.

The pancreas of the cod is diffuse. Islets of Langerhans were found in the pancreatic tissue along the bile duct as well as at the apex of the gall bladder as described by McCormick and between the pyloric caeca as described by Rennie.

INTRODUCTION

Oppel (1896), Yung and Fuhrmann (1900), and Jacobshagen (1937) refer to the account of the digestive tract of *Gadus morhua* by Thesen (1890), who describes some aspects of the histology of the tract, such as the glands in the intestine; and McCormick (1924) and Rennie (1903) describe the positions of the islets of Langerhans. We felt that further work was necessary on the morphology of the tract as a preliminary to a histochemical study.

MATERIALS AND METHODS

Live cod, held in seawater tanks at the Halifax Laboratory, were anaesthetized with tricaine methanesulfonate (Kent Chemicals Ltd., Vancouver, B.C.). Portions of the gastrointestinal tract were cut out with a razor blade and were placed immediately in a fixative.

The fixatives used were Zenker-acetic and Zenker-formol, described by Humason (1962), and 2% glutaraldehyde or 4% formaldehyde in a 0.05 mM sodium phosphate buffer at *p*H 7.2.

Some of the Zenker-acetic fixed material was dehydrated to 95% ethanol and embedded in the polyester wax introduced by Steedman (1957). The cellular detail obtained was excellent, but the wax was so soft that compression of the sections during cutting could only be prevented by sectioning in a cryostat at −4 C; and the wax tended to break up when the sections were floated out on water, resulting in separation of the tissue layers. Most of

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the material was dehydrated, cleared in benzene, embedded in Paraplast (Canlab, Montreal), and sectioned at 7μ. Stains used included Harris' haematoxylin and eosin, Mallory's trichrome, and Heidenhain's Azan methods. The presence of mucus was demonstrated by the alcian blue method for acid carbohydrate, and the technique of Humason and Lushbaugh (1960) was used for reticulin, collagen, and elastin. The aniline blue staining in the latter technique was found to be unsatisfactory, so after staining in orcein the slides were first rinsed in 70% alcohol then distilled water, and finally stained for 5 min in 0.5% aniline blue in distilled water with 5% phosphotungstic acid. The slides were rinsed in distilled water, left in 1% acetic acid in water 5 min, transferred to 95% alcohol, dehydrated, cleared, and mounted.

Most of the stains used have been described by Humason (1962): Exceptions are those used for the pancreas, which were Gomori's chromium haematoxylin phloxine technique (Greep, 1954), Gomori's azan technique (Cowdry, 1943), the alcian blue method for acid mucopolysaccharides, and the periodic acid–Schiff reaction (PAS) described by Pearse (1960).

RESULTS

Gross Morphology of the Digestive Tract

The roof and floor of the buccal cavity and pharynx are illustrated in Fig. 1 and 2. The first gill cleft is taken as the dividing line between the buccal cavity and pharynx. Longitudinal mucosal folds can be seen on the roof, and the barbel can be seen out of focus below the ventral lip. Numerous teeth are present on the premaxilla, prevomer, and dentary. In the pharynx are two dorsal convex cartilaginous plates which fit into two smaller ventral concave plates. The front of each ventral tooth plate is just behind the last gill arch. These plates are covered with sharp, conical teeth similar to those on the premaxilla and dentary. The muscles of the head of the cod have been described by Saunders and Manton (1951), except for those inserted in the pharyngeal plates. Muscles run ventrally from just inside the origin of the dilator, adductor, and levator operculi on the otic ridge to the dorsal plates. A muscle also runs anteriorly from the vertebral column to insert in each dorsal plate. Four muscles insert in each ventral plate; two muscles originate from the cleithrum, one from the hyoid, and one connects the two plates.

The tongue is supported by the hyoid which is connected to the mandible by the geniohyoideus muscle, and to the cleithrum by the sternohyoideus muscle.

Figure 3 shows the gut as it lies in the body cavity. Figure 4 shows the gonads and urinary sinus removed, and the liver displaced, and Fig. 5 and 6 show the gut removed from the body cavity with some mesenteries cut so that it can be better displayed.

The short, wide oesophagus is not well differentiated externally from the curved stomach (Fig. 5). The stomach is intermediate between the siphonal and caecal types described by Grassé (1958). There is a pouch at the elbow

joint between the main cardiac portion of the stomach and the distal pyloric portion. The latter narrows to enter the ileum. About 300 pyloric caeca join to enter the anterior end of the ileum by five or six openings. The bile and pancreatic ducts open into the intestine just distal and slightly to the right of the pyloric caeca openings (Fig. 5, 6, 7). The bile duct passes along the right side of the stomach from the gall bladder. Posterior to the entrance of the bile duct the intestine forms a loop distally, then passes forward and forms a second loop. Just posterior to the second loop the intestine widens to form a rectum, which leads into the cloaca anterior to the point of entrance of the genital and excretory ducts.

The mucosa of the oesophagus, stomach, and intestine exhibits characteristic folds (Fig. 7). The fine reticulate folds of the oesophagus change abruptly to coarse folds in the stomach. The ileum has five or six longitudinal folds, while the internal surface of the rectum has a reticulate appearance. A single flap of tissue forms a valve between the ileum and rectum. The ileum widens at the point of entry of the pyloric caeca, and the entry of the bile and pancreatic ducts appears as a whitish area.

Microscopic Anatomy of the Digestive Tract

Histologically the digestive tract can be divided into three main portions: the mouth and oesophagus; the stomach; and the pyloric caeca and intestine. The pancreas, gall bladder, and bile duct are described separately.

Mouth and Oesophagus

The mouth and oesophagus (Fig. 8–14) are lined with a stratified squamous epithelium, in which are goblet cells and taste buds (Fig. 8, 9, 10, 11). The contents of the goblet cells stain strongly with alcian blue and PAS. The epithelium on the tongue is thinly keratinized. The taste buds are supported on papillae of connective tissue, and tend to occur on the tops of ridges or folds, except on the tongue, which has a flat surface. Near the junction of the oesophagus and stomach mucus cells become more numerous and the taste buds become smaller and fewer. A taste bud from the tongue is shown in Fig. 9. The sensory cells have basal nuclei and taper distally to protrude through the epithelium. Beneath the nuclei of the sensory cells can be seen nuclei of rounded cells, which do not reach the epithelial surface.

The basal cells of the stratified epithelium (Fig. 11) are columnar. The epithelial cells become more flattened as they reach the surface, and their shape is distorted by the goblet cells.

Peripheral to the stratified epithelium is a layer of densely packed connective tissue fibres. These are especially well oriented, parallel to the surface, in the mouth. Most of these fibres stain with aniline blue so are collagenous. A few less well oriented elastic fibres are also present. Adjacent to this dense layer is a looser layer of connective tissue, with fewer fibres and more ground substance. Striated muscle occurs peripheral to the connective tissue where jaw muscles are present, such as the levator palatini in the posterior part of the

roof of the buccal cavity, and the geniohyoideus anterior to the tongue. No muscle is present in the tongue, but thick bundles of randomly oriented collagen fibres and an extensive network of elastin fibres are found in the loose connective tissue layer (Fig. 8). The oesophagus has a somewhat incomplete inner longitudinal and thicker outer circular layer of striated muscle (Fig. 10). The serosa consists of a thin layer of connective tissue, covered by a single layer of squamous cells.

STOMACH

There is a sharp contrast in the histology of the oesophagus and stomach (Fig. 12–20), and the changeover is abrupt (Fig. 12, 13, 14). The stratified squamous epithelium of the oesophagus is replaced in the stomach by tall columnar epithelial cells, and compound glands are present (Fig. 13). The connective tissue layer is less compact than in the oesophagus. The collagenous fibres are denser immediately under the epithelium, and this is the only distinction between the mucosal and submucosal regions. The muscularis mucosae is composed mainly of longitudinal fibres, and varies considerably in extent, becoming well defined only in the pyloric part of the stomach. The circular striated muscle layer of the oesophagus is gradually replaced by a circular smooth muscle layer in the stomach, and the inner longitudinal striated muscle of the oesophagus disappears in the stomach, where an outer longitudinal smooth muscle layer appears (Fig. 14).

The layers present in the cardiac part of the stomach are shown in Fig. 15. The columnar epithelial cells (Fig. 16) have a clear border which is more eosinophilic than the rest of the cytoplasm and stains purple with azan stain. The alcian blue and PAS techniques stain a mass of granules in the border and between the nucleus and the border. The free edge of the border is indented and appears red with azan stain. There is a prominent thickening between the cells in the region of the border and a fibrous area both below the border and along the cell junctions, tapering off opposite the nucleus. This region, which stains red, is possibly part of the cell web, which plays a supporting role in the cell (Leblond et al., 1960; Ham, 1965). The cells of the compound tubular glands are of one type only, with eosinophilic granules. The change from lining epithelial to secretory cells at the mouth of the glands is gradual; the clear border is lost and granules appear in the cells (Fig. 17). Among the columnar epithelial cells and in the connective tissue just beneath them are found rounded cells containing mainly eosinophilic granules (Fig. 16).

At the pyloric end of the stomach (Fig. 18) the circular muscle layer becomes well developed to form a sphincter. The compound tubular glands are lost, but the epithelial folds become deeper, forming tubules which branch, giving a glandular appearance. However, the cells in these tubules do not contain granules like the compound gland cells; they stain with alcian blue (Fig. 19), although less intensely than the surface epithelial cells (Fig. 20), whose indented border stains very deeply.

INTESTINE AND PYLORIC CAECA

The tissue layers of the ileum are shown in Fig. 21 and 22 of the rectum in Fig. 23, and of the pyloric caeca in Fig. 24 and 25. Thesen (1890) states that there are no glands in the pyloric caeca, but we found that these three regions are similar, although the proportions of the different layers vary. The lining epithelial cells of the ileum and rectum are mainly simple columnar. At the free surface of the cell is a "brush" border (Fig. 26 and 27) except in the rectum where the cells at the apices of the folds often appear to be covered with long cilia-like fibres (Fig. 28). It is difficult to determine with the light microscope whether the brush and long fibres are microvilli or cilia. Thesen describes cilia as being on the epithelial cells of the pyloric caeca. A dark band which can be seen below the epithelial border could represent the region of basal bodies and striated rootlets, but could also be an aggregate of the cell web. An electron microscopic study has been carried out to establish the nature of the border (Odense and Bishop, 1966).

Glands open into the intestinal cavity at intervals, as described by Thesen. These are composed of rounded cells which stain more darkly with haematoxylin than the columnar cells, and lack the brush border. Alcian blue- and PAS-staining goblet cells are present in the glands, and among the epithelial cells near the openings of the glands (Fig. 29). They are usually as tall as the epithelial cells and are dilated with mucus distally. The proximal part of the cell is narrow and contains a nucleus at the same level as the nuclei of the adjacent columnar cells. The goblet cells are smaller in the fundus of the glands, and appear to extrude their contents only when among the columnar cells. Those farthest away from the mouths of the glands have lost most of their mucus content, a fact which suggests that they are constantly replenished from those in the glands. The columnar cells farthest away from the gland openings seem to be effete. Al-Hussaini (1949) noted a similar regeneration of mucus cells from the bases of the epithelial folds in the three cyprinids that he studied.

Cells similar to the "pear-shaped" cells of Al-Hussaini (1949) are found in both the lining epithelium and glands (Fig. 30 and 31). These cells are approximately half the height of the columnar cells, with one end extending to the free border. The oval nucleus is basal and several rows of eosinophilic granules run from this level to the apex of the cell. These cells are not stained by the alcian blue or PAS techniques.

The connective tissue layer is of the ordinary loose type, and consists mainly of collagenous fibres with a few elastic fibres. This layer is very narrow in the pyloric caeca. No muscularis mucosa is present, and there is no distinct lamina propria.

Peripheral to the connective tissue is a thick inner layer of circular, smooth muscle and thinner outer layer of longitudinal muscle; the latter is very thin in the pyloric caeca. The smooth muscle of the ileorectal valve is mainly circular, and radially oriented fibres run from the base of the valve to the

longitudinal muscle coat (Fig. 32). At the cloaca the glandular mucosa is replaced by a stratified keratinized squamous epithelium.

PANCREAS

Pancreas tissue occurs between the pyloric caeca (Fig. 24, 25, 33) along the bile duct (Fig. 34) and the hepatic ducts, but does not penetrate far into the liver. It also covers the gall bladder (Fig. 37), and follows the blood vessels in the mesenteric tissue near the gut. Blood vessels are often found running from the pancreas through the wall of the pyloric caeca to the submucosa (Fig. 33). The numerous small pancreatic ducts converge into four or five larger ones which enter the ileum just anterior to the bile duct. Rennie (1903) in his study on cod described islets of Langerhans only among the caeca, while McCormick (1924) reported one principal islet at the apex of the gall bladder. We found small islets between the caeca, but most of the islet tissue is concentrated near the bile duct, and, as reported by McCormick, at the apex of the gall bladder.

There is some collagenous connective tissue around the margin of an islet but little inside. The many capillaries permeating the islet result in a coiled arrangement of cells (Fig. 34). Usually there are only one or two layers of cells between the capillaries. Two types of cell occur in the islet, one type, found mainly in the centre, has an elongate nucleus and granules which stain densely with haematoxylin. The position of the nucleus in the cell is variable. The other type of cell is somewhat larger and more rounded, and contains fewer eosinophilic granules which are not preserved in formalin-fixed material (Fig. 35). The central nucleus is usually large and round, with one distinct acentric nucleolus and little chromatin. In addition to the two predominant cell types smaller cells which stain more densely with haematoxylin occur among the larger cells; they are similar to the central cells but could also represent a different stage of activity of the larger cells. These cellular differences are accentuated by the Gomori chromium haematoxylin phloxine and Gomori's azan techniques. In the azan technique the granules of the central cells are purple and orange, and those of the outer cells pale blue (Fig. 36). There is little difference in the size of the granules. In the chromium haematoxylin phloxine technique the central cells stain deep mauve and the outer cells very pale mauve.

The exocrine cells of the pancreas in the cod are very basophilic, and the zymogen granules do not stand out clearly unless an azan method is used, when the large red zymogen granules contrast with the purple ergastoplasm.

GALL BLADDER AND BILE DUCT

The gall bladder (Fig. 37, 38) has an unfolded simple columnar epithelium resting on a dense connective tissue layer, composed mainly of collagenous fibres. Peripheral to this is a thin layer of smooth muscle which is difficult to distinguish from the connective tissue unless a collagen stain is used. The

bile duct is also lined with a simple columnar epithelium, among which are some cells with Alcian-blue-staining granules. The epithelium is surrounded by collagenous connective tissue and smooth muscle.

DISCUSSION

It is difficult to correlate the structure of the digestive tract of the cod closely with its feeding habits. The cod is mainly carnivorous, but the food is not specialized and varies with the population, the season, and the size of the individual. Powles (1958) found that in the Gulf of St. Lawrence cod 11–30 cm long fed mainly on pelagic crustaceans; whereas with increase in size, fish and bottom living invertebrates became more important food items. He also noted differences in the food of the cod in four areas studied, as well as variations over a 3-month period. Srivastava (personal communication) has studied the different types of feeding associated with the varied diet of the adult cod. She found that it usually swallows its food whole, although some manipulation can take place; for instance molluscs may be shaken free from their shells. The dentition is therefore fairly simple, with pointed, curved teeth which would help to impale a prey but which would be useless for grinding. Mechanisms for grinding the food before swallowing it are a common feature of herbivorous fish such as *Labeo horie* or *Cyprinus carpio communis*.

Srivastava also found that the cod selects its food by sight when catching food in midwater and by taste buds on the pelvic fins and barbel when bottom-feeding. The taste buds in the mouth and eosophagus are important for the more specific selection which takes place once the food has been ingested, and the cod sometimes mouths the food before swallowing it. Striated muscle in the oesophagus may make voluntary food rejection possible back to this point, and the increase of mucus-secreting cells in this region presumably lubricates the food for easier swallowing. There is no clear distinction between the sensory and sustentacular cells of the taste buds, apart from the sensory hairs of the former, so continuous replacement of the sensory cells by the sustentacular cells may take place as described by Brachet and Mirsky (1964) and Beidler and Smallman (1965).

Gastric glands are described in many fish with stomachs, for instance the bottom feeders *Mulloides* (Al-Hussaini, 1946) and *Prionotus* (Blake, 1937); but they often have wider lumina and are less densely packed than those in *Gadus*. The condition in *Gadus* is more comparable with that of the predaceous *Centropristes* (Blake, 1930). The presence of a stomach is not necessary for a carnivore; for instance the cyprinid *Gobio gobio* is largely predaceous and lacks gastric glands (Al-Hussaini, 1949); but the acid medium probably helps to break down the exoskeleton of the crustaceans, echinoderms, and others which form a large part of the diet of the cod. As in other teleosts there appears to be no cytological differentiation into oxyntic and peptic cells in the gastric glands. The presence of granular cells in the mucosa introduces the possibility of another type of secretion in the stomach.

Intestinal glands are found only in the gadoids, but a folding of the intestine, resulting in morphologically gland-like structures, occurs in *Prionotus carolinus* (Blake, 1937), giving a large surface of intestinal epithelium. Blake suggests that this may indicate a stage towards the full development of glands in which a cytological difference between the glandular and epithelial cells would also be evident. *Gadus* provides a further intermediate stage in which the glandular cells show some cytological differences, but this does not necessarily imply that they have a specific secretion. A possible enzyme source in the intestine is the "pear-shaped" cells.

The numerous pyloric caeca greatly increase the surface area of the intestine. There are slight morphological differences between the caeca and intestine, but it will only be possible to determine any differences in function by histochemical studies.

The staining reactions of the cells in the islets of Langerhans of the cod agree fairly closely to those for the other teleosts such as *Lebistes reticulatus* (Grosso, 1950) and *Thunnus thynnus* (Planas and Garcia, 1964). Falkmer and Olsson (1962) showed in a study of normal and alloxan, treated *Cottus scorpius* that only the darker staining central cells were affected by alloxan, and their ultrastructure was similar to that of mammalian beta cells. Grosso (1950) also found that the darker cells of *Lebistes* were affected by alloxan. On these bases, therefore, the central dark-staining cells of *Gadus* are beta cells, and the outer cells are alpha cells.

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NOTE: *Figures 1 to 38 on following pages.*

Explanation of Figures

Plate I

Fig. 1. Roof of mouth: PM = Teeth on premaxilla, PV = Teeth on prevomer, TP = Cartilaginous tooth plate.

Fig. 2. Floor of mouth: B = Barbel, D = Teeth on dentary, T = Tongue, TP = Cartilaginous tooth plate.

Plate II

Fig. 3. Left body wall removed to display the alimentary canal in its natural position in the body cavity.

Fig. 4. Liver displaced and gonads and urinary sinus removed: CL = Cloaca, CS = Cardiac portion of stomach, I = Ileum, L = Liver, O = Oesophagus, PC = Pyloric caeca, PS = Pyloric part of stomach, R = Rectum, S = Spleen.

Plate III

Fig. 5. Alimentary canal from oesophagus to cloaca, excised from the body cavity.

Fig. 6. Drawing of part of the alimentary canal to illustrate the bile and hepatic ducts more clearly. Pancreatic tissue and blood vessels have been removed from round these ducts: BD = Bile duct, CD = Cystic duct, CS = Cardiac part of stomach, HD = Hepatic duct, I = Ileum, L = Liver, O = Oesophagus, P = Pancreas, PC = Pyloric caeca, PS = Pyloric part of stomach, R = Rectum.

Plate IV

Fig. 7. Internal surface of alimentary tract from oesophagus to anterior ileum following middorsal incision: MI = Mucosal folds of ileum, MO = Mucosal folds of oesophagus, MS = Mucosal folds of stomach, OBD = Opening of bile duct, OPC = Opening of pyloric caeca, PS = Pyloric sphincter.

Plate V

Fig. 8, 9 Photomicrographs of longitudinal section of tongue. Specimen fixed in buffered 4% formaldehyde, embedded in Paraplast, sectioned at 7μ, and stained with Harris' haematoxylin and eosin: C = Connective tissue containing densely packed collagen fibres, CE = Connective tissue containing loosely arranged collagen and elastic fibres, P = Papilla of connective tissue supporting taste bud, S = Sensory cells of taste bud, SE = Stratified squamous epithelium, ST = Sustentacular cells, TB = Taste bud.

Fig 10. Photomicrograph of longitudinal section of oesophagus. Specimen fixed in bufferen 4% formaldehyde embedded in Paraplast, sectioned at 7μ, and stained in Harris' haematoxylid and eosin: CM = Circular striated muscle layer, CT = Connective tissue, LM = Longitudinal striated muscle layer, MC = Mucus cells, SER = Serosa.

Fig. 11. Photomicrograph of longitudinal section of oesophagus. Specimen fixed in Zenker acetic fixative, embedded in polyester wax, sectioned at 5μ, and stained in Harris' haematoxylin and eosin: E = Columnar embryonic epithelial cells, MC = Mucus cell.

Plate VI

Fig. 12. Drawing of longitudinal section of area of transition from oesophagus to stomach: stomach to the left of drawing, oesophagus to the right. Regions shown in Fig. 13, 14 are indicated. Section fixed in 4% buffered formol saline, embedded in Tissuemat, sectioned at 5μ, and stained in Harris' haematoxylin and eosin.

Fig. 13. Photomicrograph of region of transition from oesophageal to gastric epithelium; CO = Columnar epithelium, LM = Longitudinal striated muscle layer, SE = Stratified squamous epithelium, T = Compound tubular glands.

Fig. 14. Photomicrograph of region of transition from striated to smooth muscle: CSM = Circular smooth muscle layer, LSM = Longitudinal smooth muscle layer, CM = Circular striated muscle layer.

Plate VII

Fig. 15. Photomicrograph of longitudinal section of middorsal cardiac part of stomach. Specimen fixed in 4% buffered formalin, embedded in Paraplast, sectioned at 7μ, and stained with Harris' haematoxylin and eosin.

Fig. 16, 17. Photomicrographs of mucous membrane of longitudinal section of cardiac part of stomach. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Heidenhain's "Azan" stain: CB = Clear border, CO = Columnar epithelium, CSM = Circular smooth muscle layer, LSM = Longitudinal smooth muscle layer, MM = Muscularis mucosae, RC = Round cells containing granules, T = Compound tubular glands.

Plate VIII

FIG. 18. Photomicrograph of longitudinal section of pyloric part of stomach. Specimen fixed in Zenker formol, embedded in Paraplast, sectioned at 7μ and stained with Harris' haematoxylin and eosin.

FIG. 19, 20. Phase contrast photomicrographs of pyloric region of stomach. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with alcian blue. Fig. 19 shows cells in a tubule, and Fig. 20 lining epithelial cells: CO = Columnar epithelium, CT = Connective tissue, IB = Indented border, MM = Muscularis mucosae, T = Tubule.

FIG. 21, 22. Photomicrographs of transverse section (Fig. 21) and longitudinal section (Fig. 22) of ileum. FIG. 23. Photomicrograph of longitudinal section of rectum. Fixed in buffered 4% formaldehyde, embedded in Paraplast, sectioned at 7μ, and stained with Harris' haematoxylin and eosin: CSM = Circular smooth muscle layer, CT = Connective tissue, E = Epithelium, LSM = Longitudinal smooth muscle layer, TG = Compound tubular glands.

Plate IX

FIG. 24. Photomicrograph of transverse section of pyloric caeca. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Delafield's haematoxylin and eosin.

FIG. 25. Photomicrograph of longitudinal section of pyloric caeca. Specimen fixed in buffered 4% formaldehyde, embedded in Paraplast, sectioned at 7μ, and stained with Harris' haematoxylin and eosin: CSM = Circular smooth muscle layer, E = Epithelium, LSM = Longitudinal smooth muscle layer, P = Pancreas, TG = Compound tubular glands.

FIG. 26, 27, 28. Photomicrographs of surface epithelial cells from ileum (Fig. 26), pyloric caecum (Fig. 27) and rectum (Fig. 28). Specimens fixed in Zenker acetic, embedded in polyester wax, and sectioned at 2μ. Pyloric caecum and rectum stained with Heidenhahn's iron haematoxylin, ileum stained with Heidenhahn's azan stain: B = "Brush border," F = Interlacing cilia-like fibres, G = Goblet cell.

Plate X

FIG. 29. Phase photomicrograph of longitudinal section of ileum. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with alcian blue.

FIG. 30. Photomicrograph of longitudinal section of ileum, showing surface epithelium. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Harris' haematoxylin and eosin: E = Effete columnar epithelial cells, G = Goblet cells, S = Striated cell.

Plate XI

FIG. 31. Photomicrograph of longitudinal section of ileum, showing the compound tubular gland region. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Harris' haematoxylin and eosin: G = Goblet cell, S = Striated cell.

FIG. 32. Drawing of ileorectal valve: CSM = Circular smooth muscle layer, LSM = Longitudinal smooth muscle layer, M and SM = Mucosa and submucosa, R = Radially oriented smooth muscle, V = Valve.

Plate XII

FIG. 33. Photomicrograph of longitudinal section of pyloric caeca. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Harris' haematoxylin and eosin: BV = Blood vessel, P = Pancreas, T = Compound tubular gland.

FIG. 34, 35. Photomicrographs of exocrine and endocrine pancreas near bile duct.

FIG. 34. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Harris' haematoxylin and eosin.

FIG. 35. Specimen fixed in buffered 4% formaldehyde, embedded in Paraplast, sectioned at 7μ, and stained with Harris' haematoxylin and eosin: α = Alpha cells, β = Beta cells, E = Exocrine cells.

Plate XIII

FIG. 36. Photomicrograph of Islet of Langerhans in pancreas near bile duct. Specimen fixed in Zenker acetic, embedded in polyester wax, sectioned at 5μ, and stained with Gomori's "azan" stain: α = Alpha cell, β = Beta cell, E = Exocrine cell.

FIG. 37. Photomicrograph of section of wall of gall bladder.

FIG. 38. Photomicrograph of longitudinal section of bile duct.

FIG. 37, 38. Specimens fixed in buffered 4% formaldehyde, embedded in Paraplast, sectioned at 7μ, and stained with Harris' haematoxylin and eosin: C = Simple columnar epithelium, P = Pancreas.

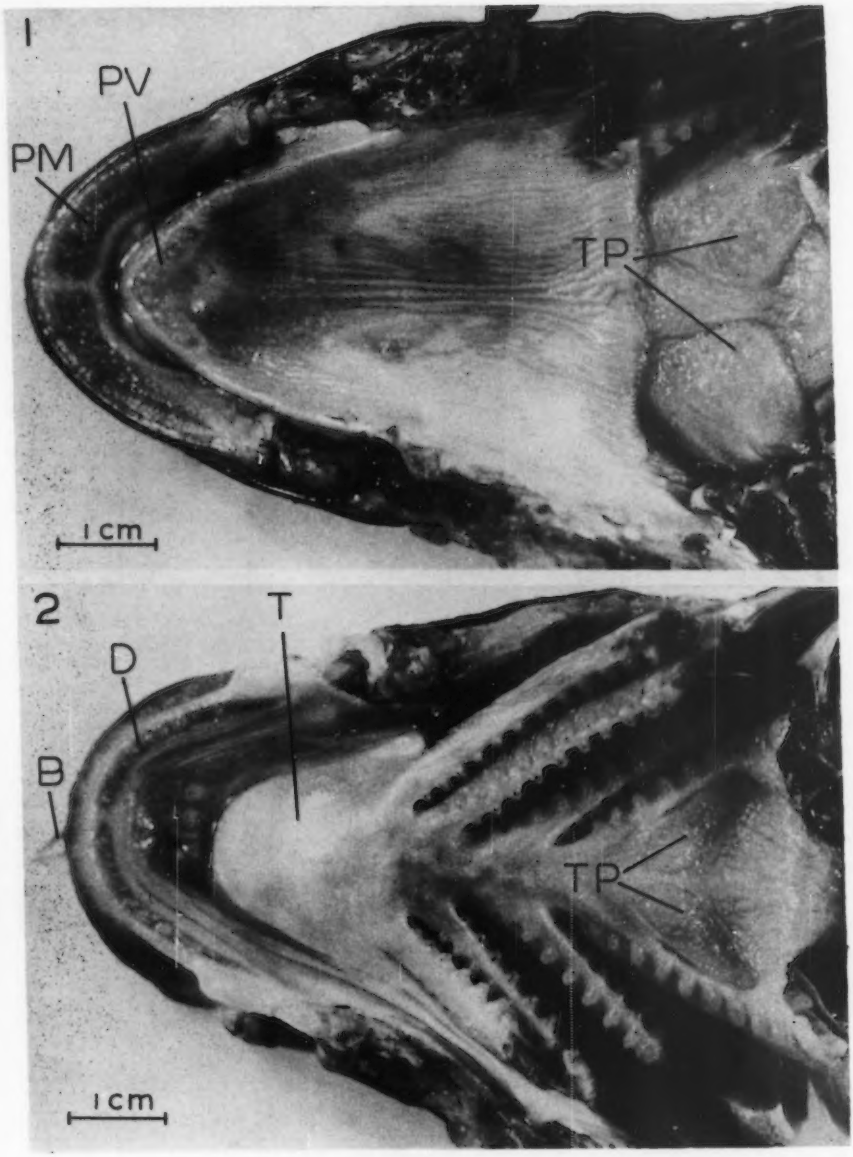

Plate I

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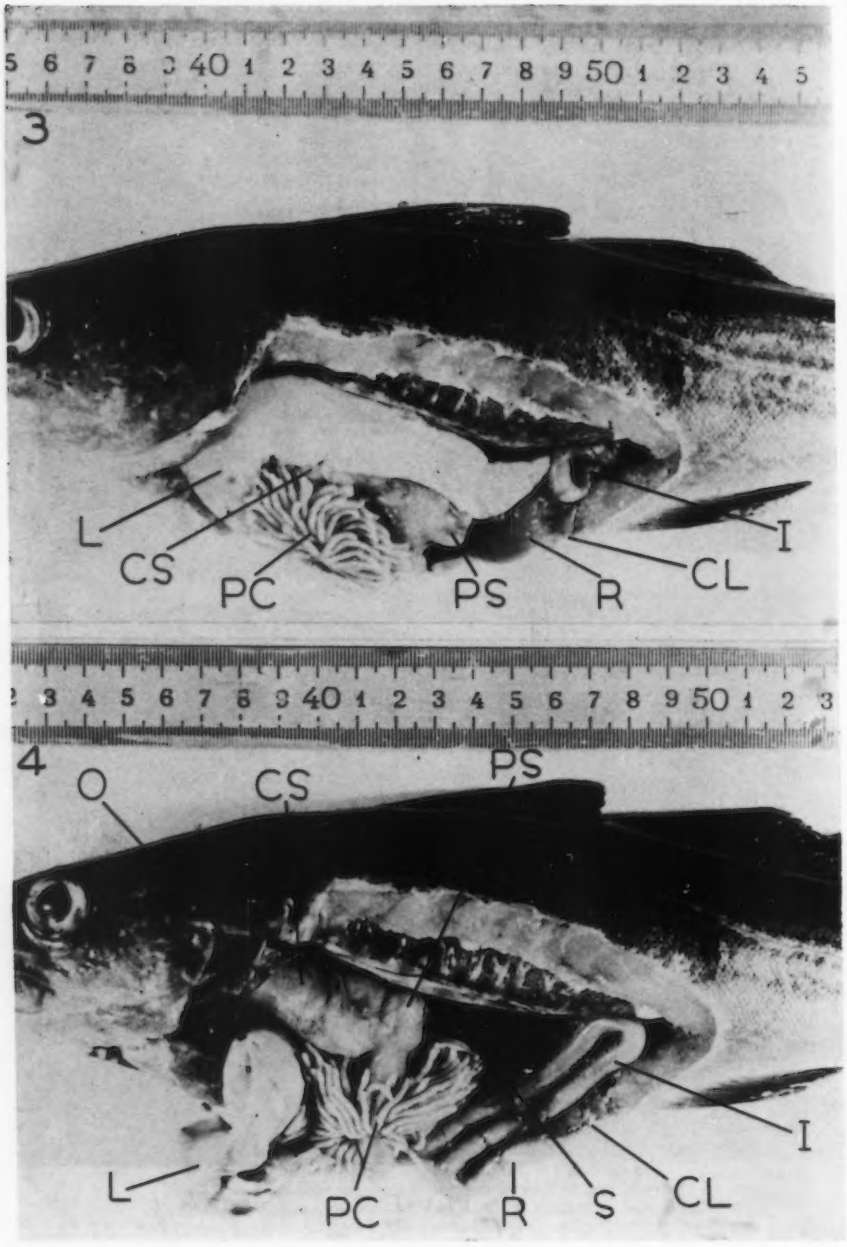
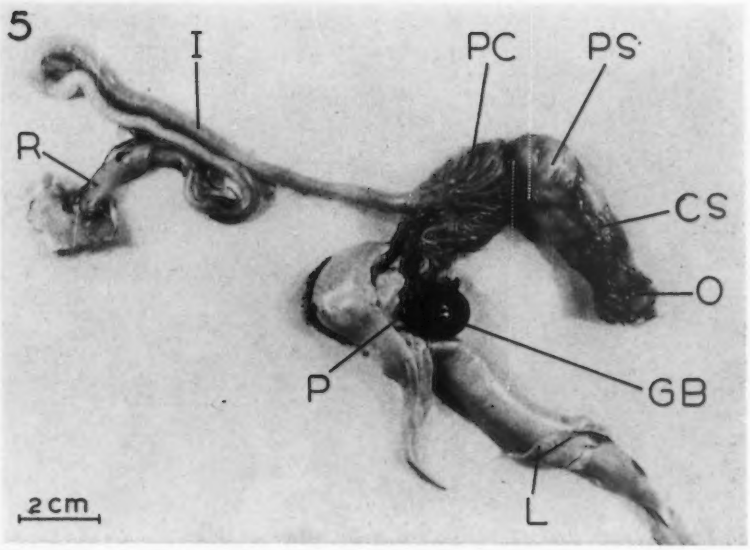


Plate II

Bishop and Odense — J. Fish. Res. Bd. Canada



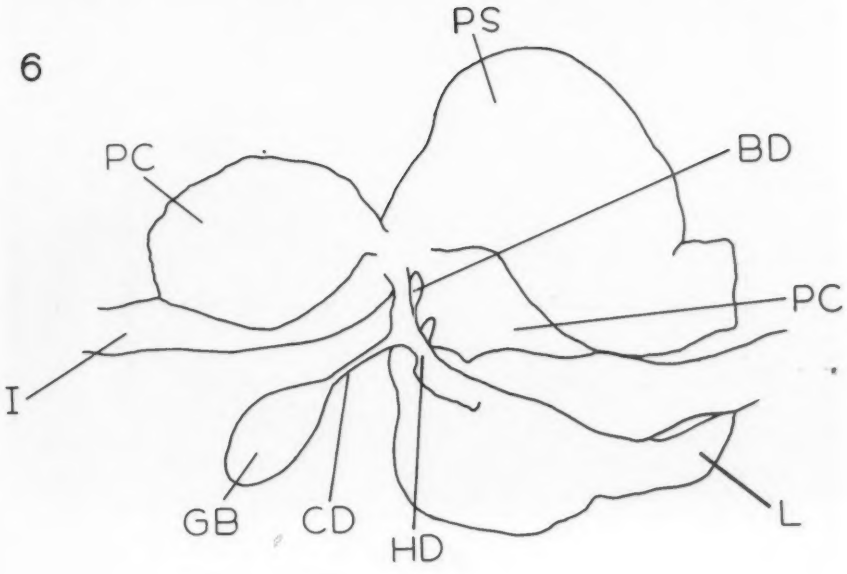


Plate III

Bishop and Odense — J. Fish. Res. Bd. Canada

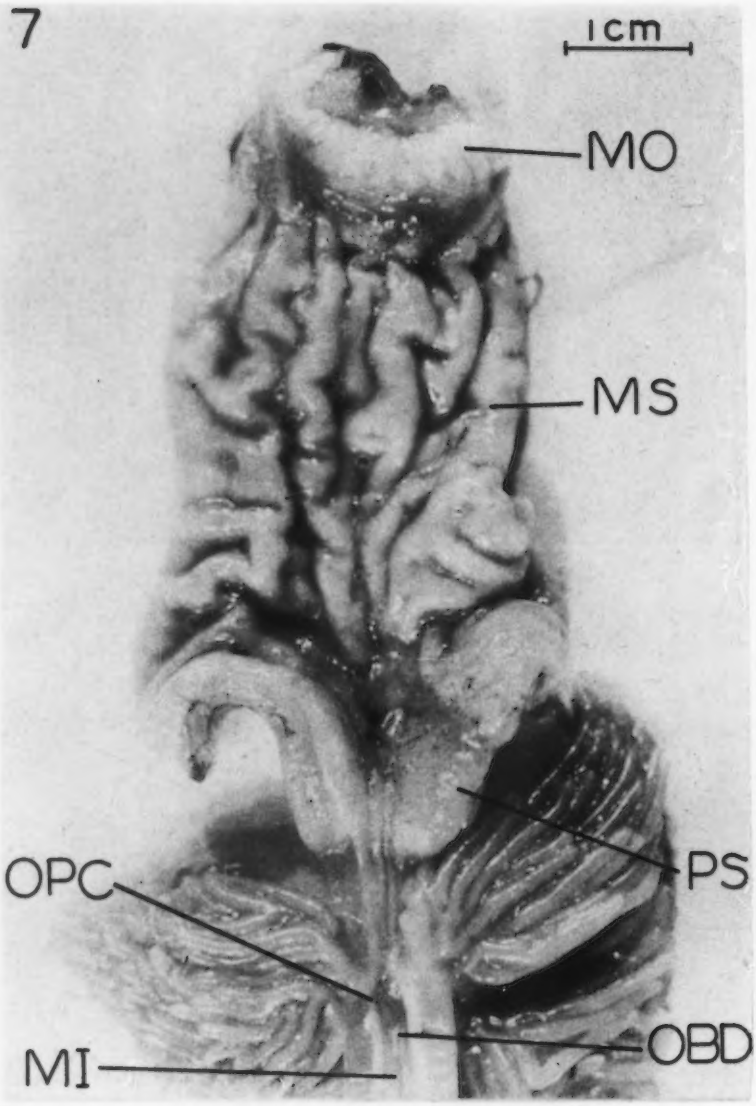


Plate IV

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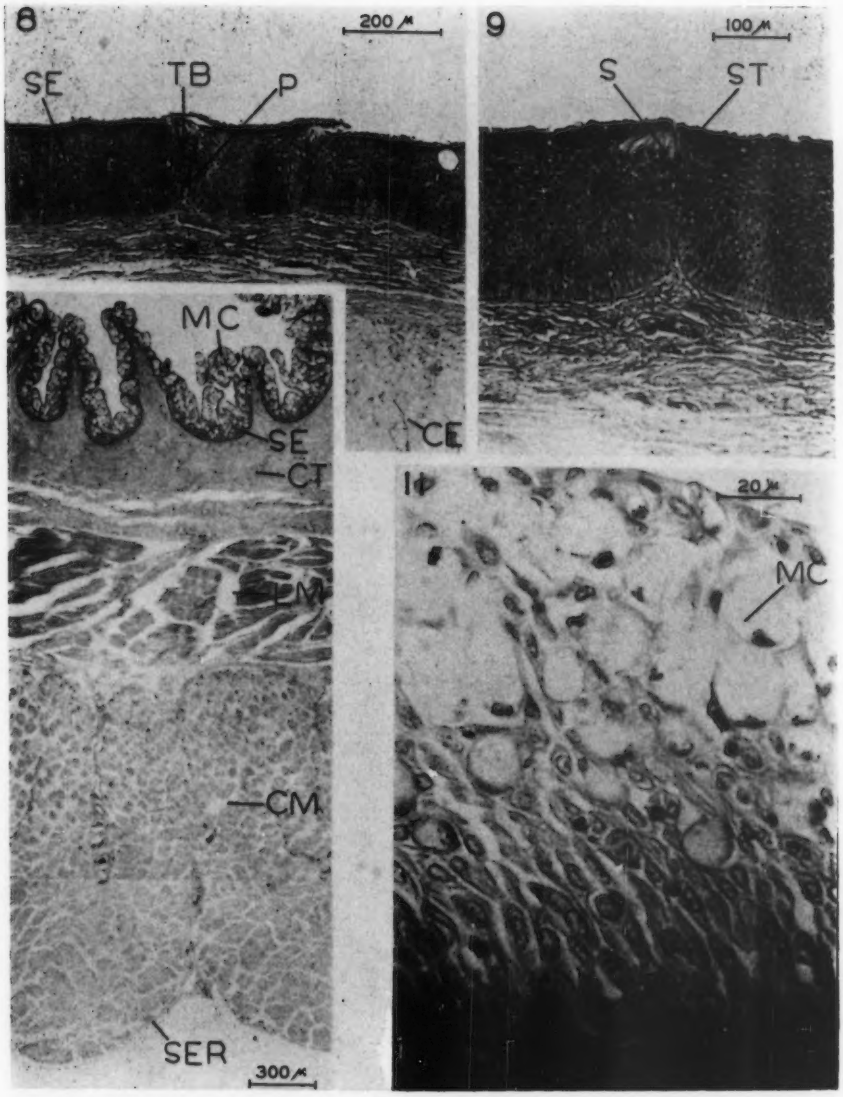

Plate V

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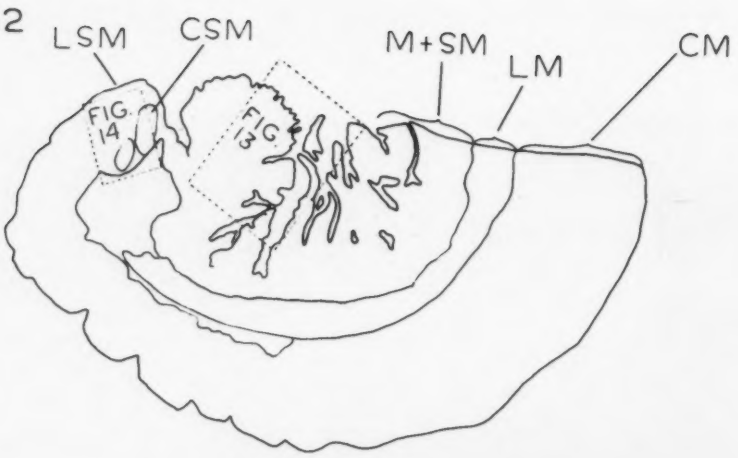

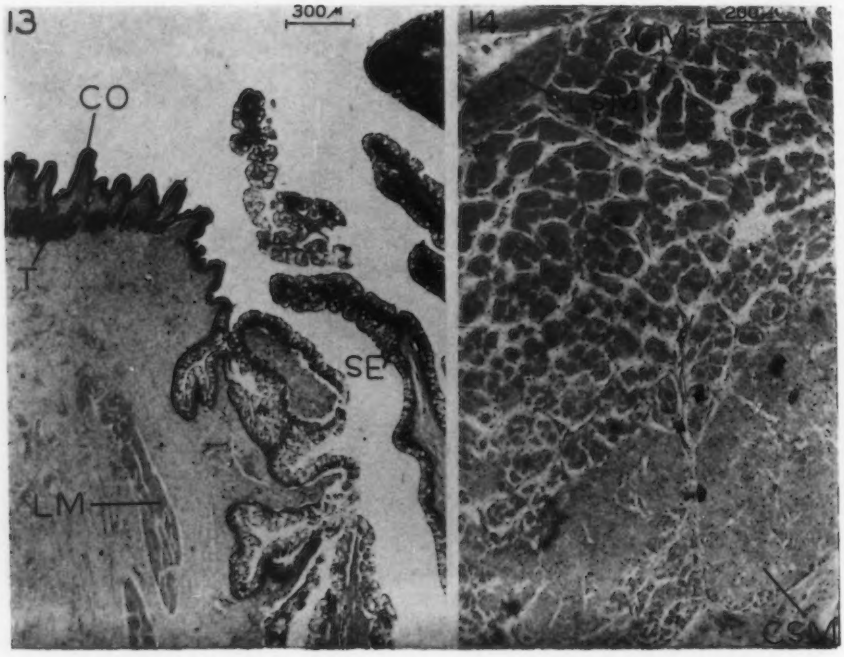

Plate VI

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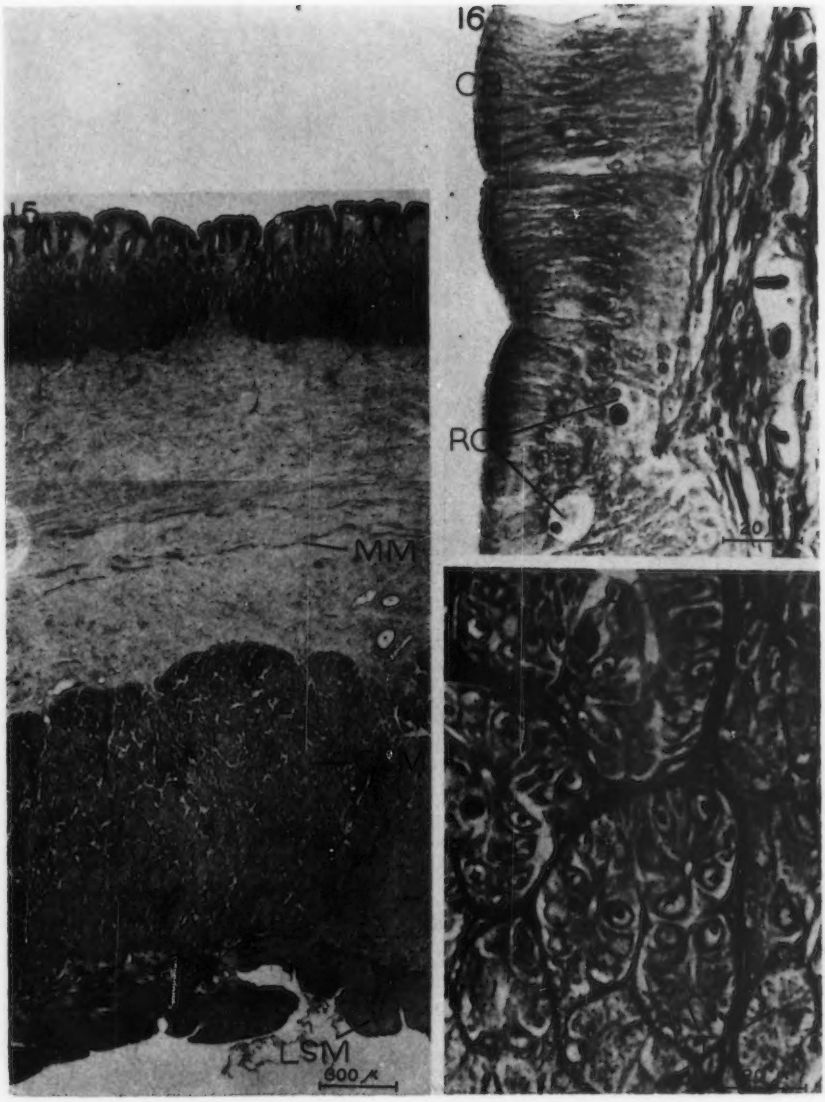

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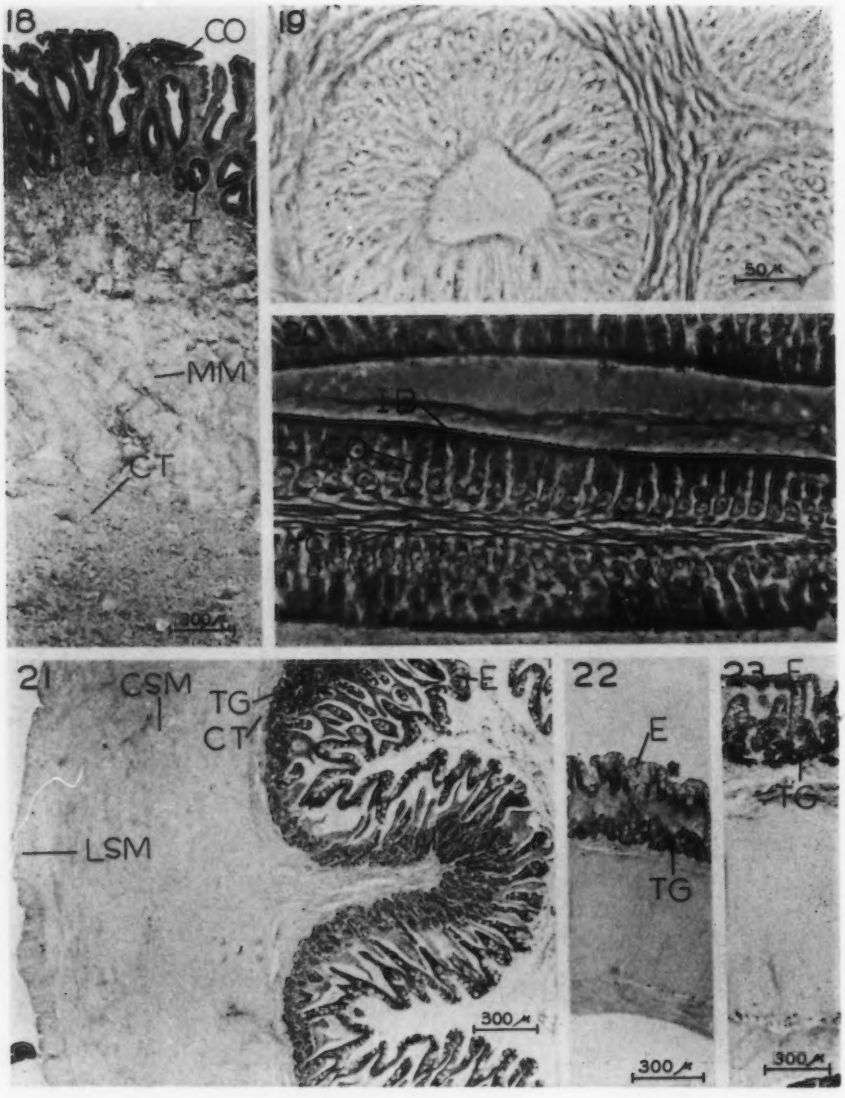

Plate VIII

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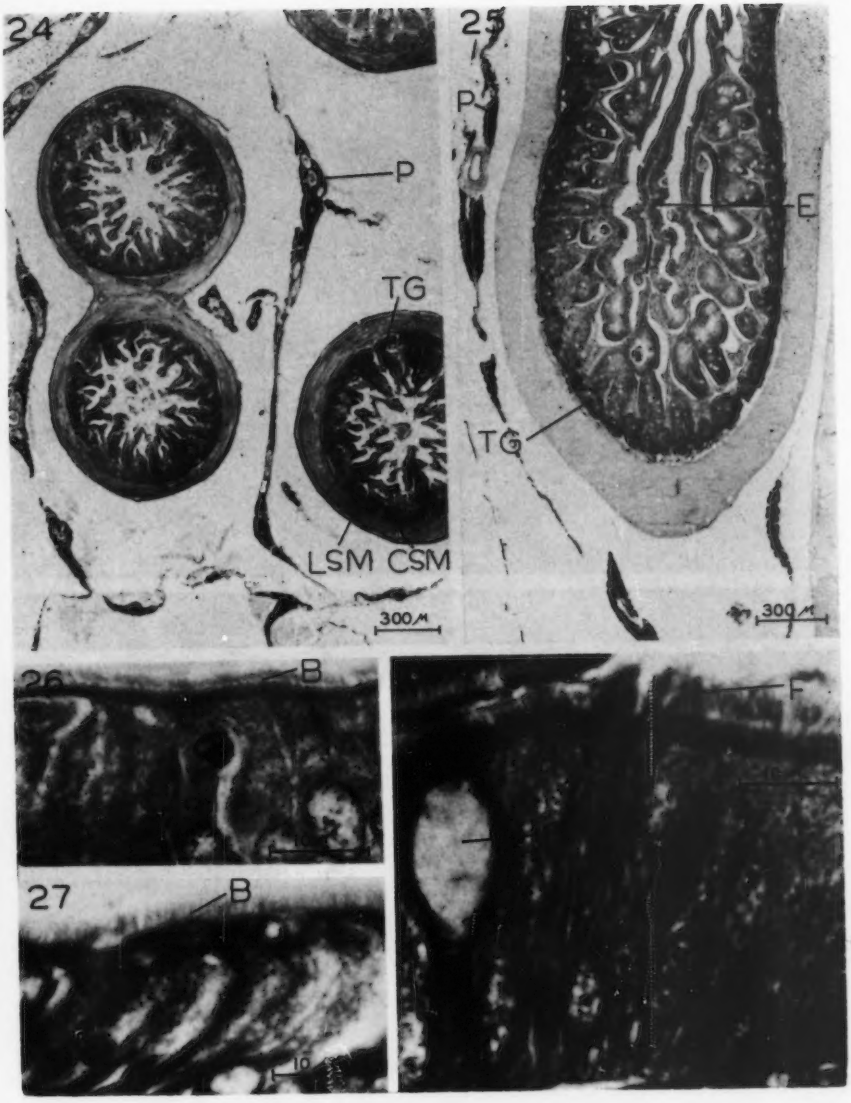

Plate IX

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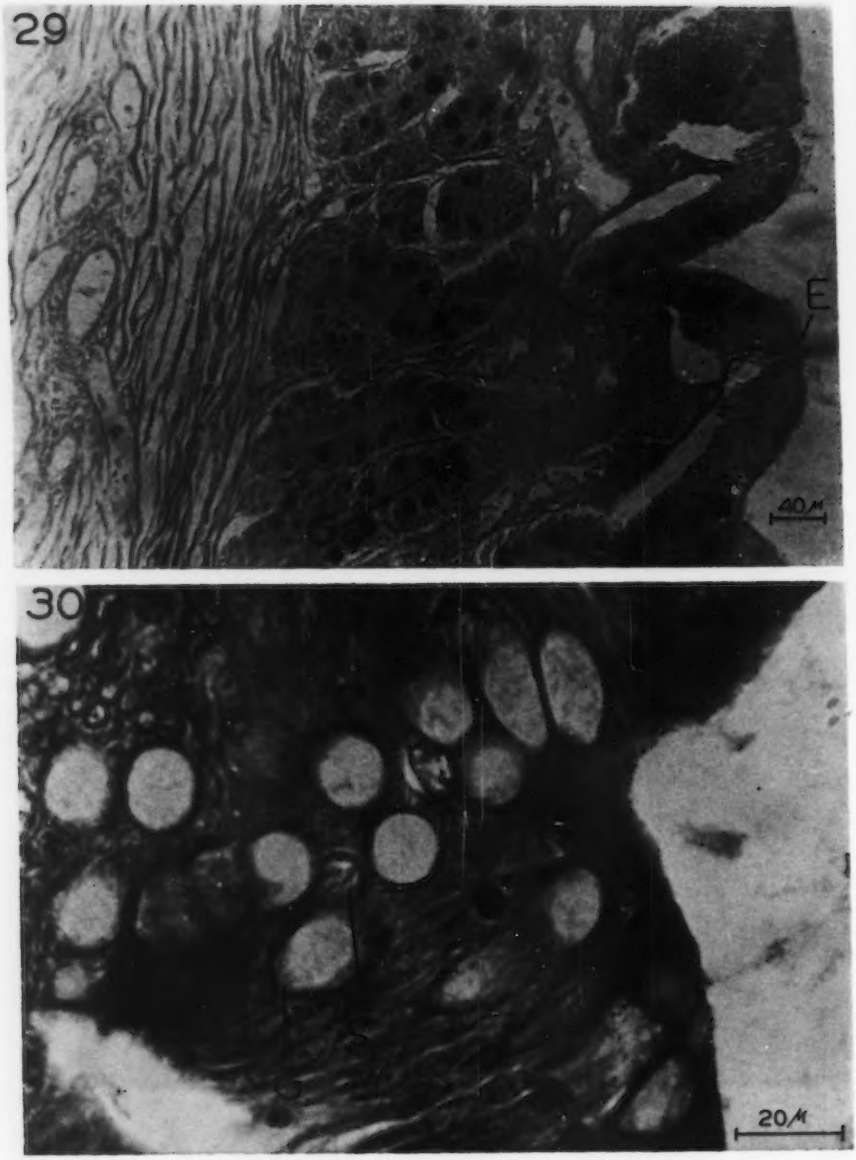

Plate X

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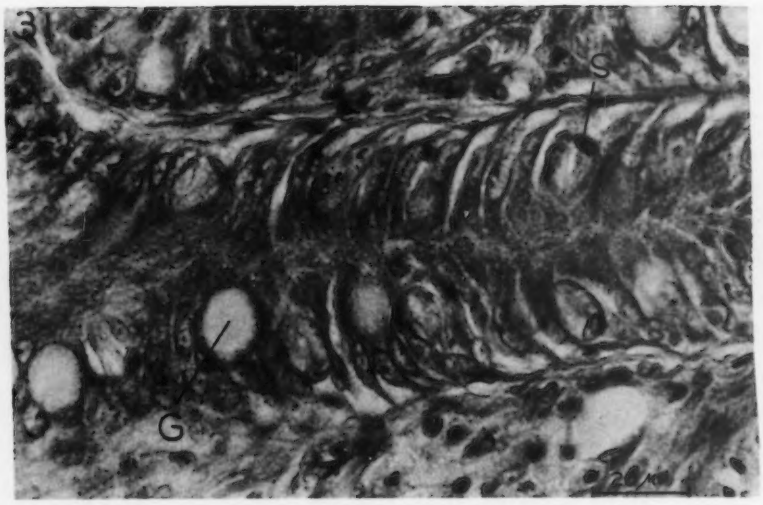

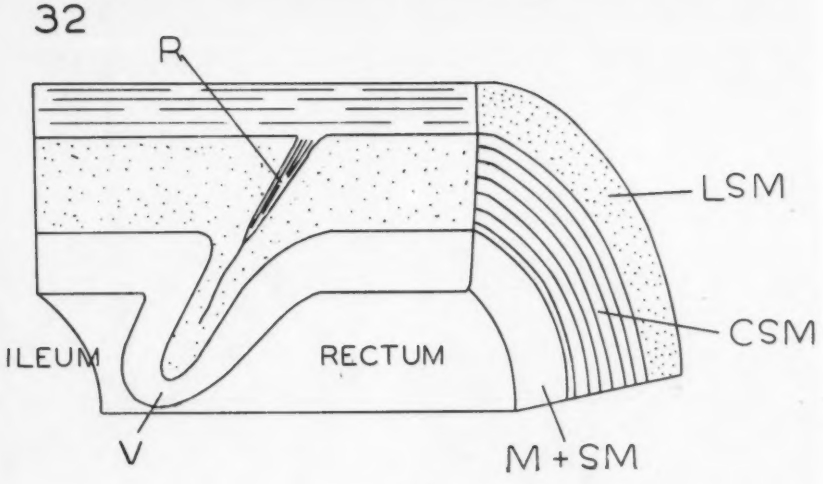

Plate XI

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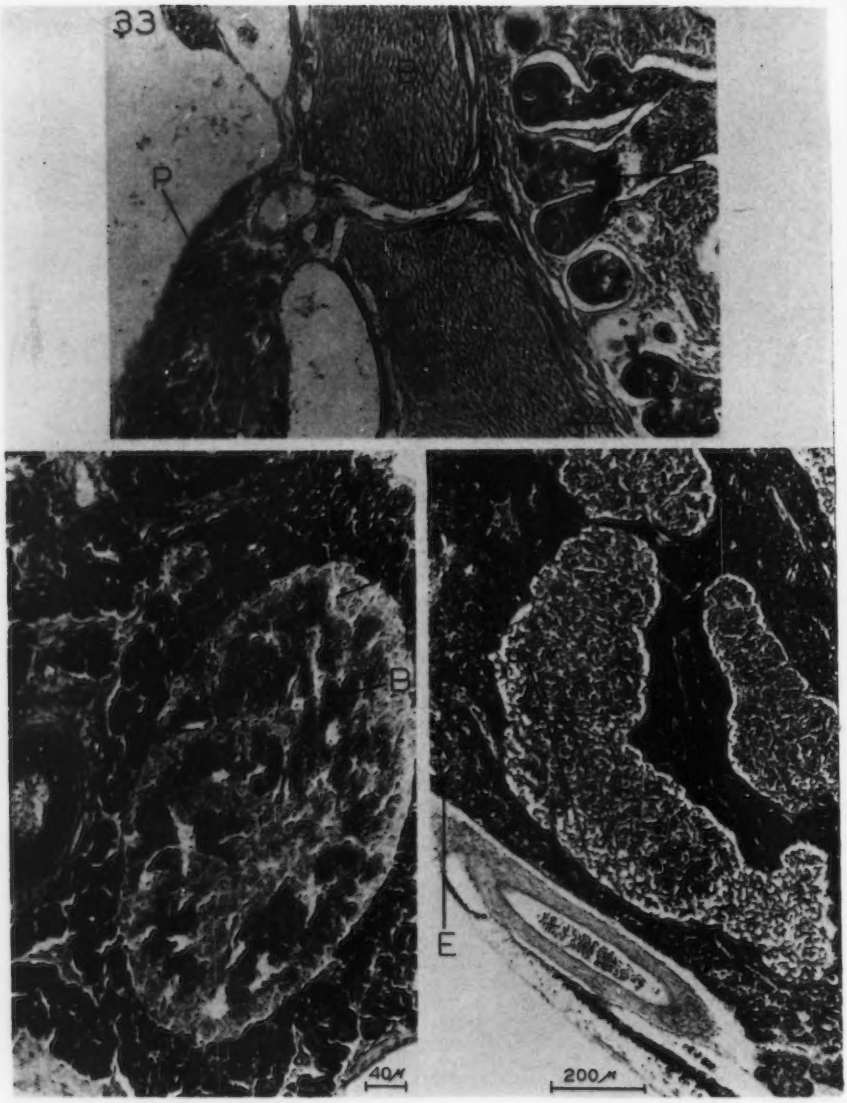

Plate XII

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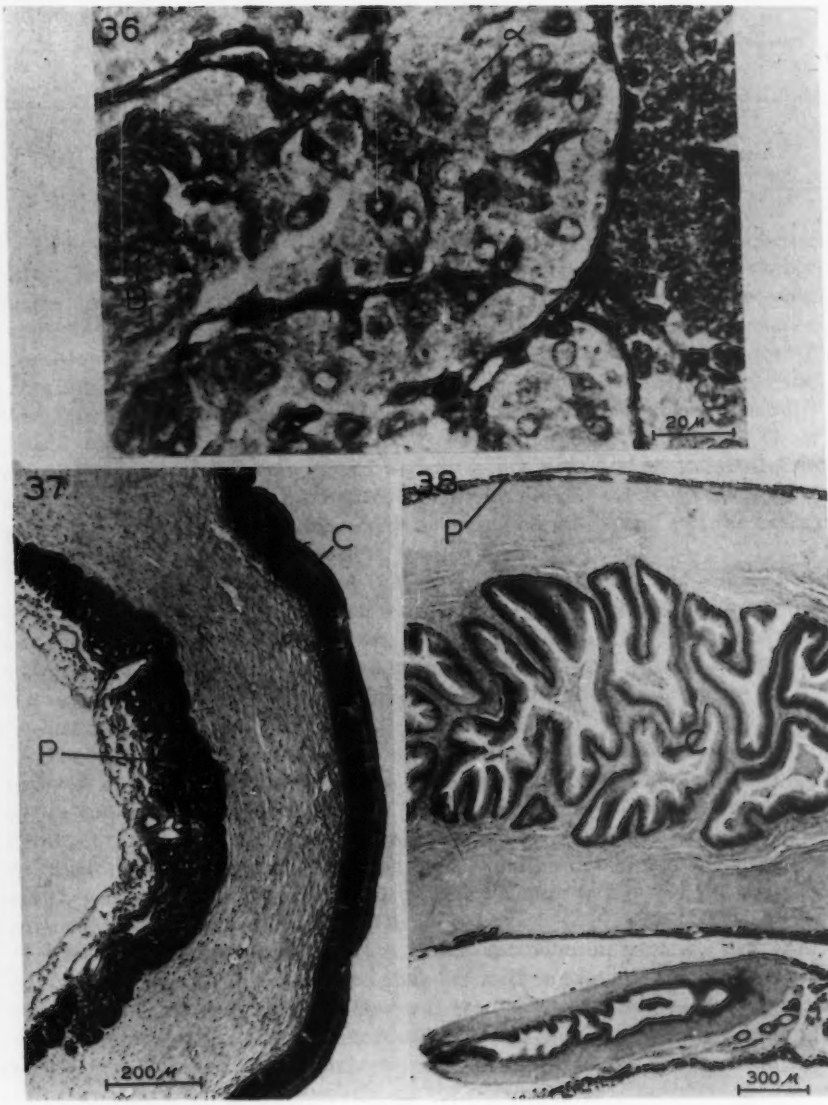

Plate XIII

Bishop and Odense — J. Fish. Res. Bd. Canada

NOTES

Adjustment of Buoyancy in Atlantic Salmon Parr in Relation to Changing Water Velocity

Fishes living in rapidly flowing water must expend energy to resist being swept downstream. As current speed increases, energy demands presumably become greater and the tendency for downstream displacement becomes greater. Among the adaptations in stream-inhabiting fishes for maintenance of position in a current is an increase in body density through reduction of the swimbladder volume. Saunders (1965) demonstrated that Atlantic salmon parr (*Salmo salar* Linnaeus) and brook trout (*Salvelinus fontinalis* (Mitchill)) respond to changes in water velocity by adjusting their buoyancy. Those held in flowing water became less buoyant (more dense) than those held in still water. This work has been extended in the present study to investigate the time for adjustment of buoyancy to changing current speed and the extent of adjustment over a range of current speeds.

Atlantic salmon yearling and underyearling parr were obtained in September and October from the East Florenceville Fish Culture Station, New Brunswick. They were held indoors in running aerated water in stainless steel, fibreglass or wooden tanks and were fed ground liver at least 5 days out of seven. The water temperature ranged from 14 C in September to 7 C in late December.

The buoyancy was measured in a specially constructed flume which consisted of a pair of plexiglass chambers through which different water velocities could be directed (Saunders, 1965). After turning off the water flow it was possible to make these chambers airtight by removing the screens from their ends and replacing them with gasketed plexiglass doors. The chambers then could be partly evacuated using a vacuum pump and the pressure in the chamber read from a manometer.

Parr were anaesthetized with tricaine methanesulphonate (M.S. 222) and two parr placed in each flume chamber with a negligible flow. Upon recovery from the anaesthetic the flow was gradually increased to the required velocity and maintained for the duration of the test. The flow was then stopped, the fish anaesthetized, the chambers made airtight and the pressure reduced until the anaesthetized fish just rose off the bottom. The atmospheric pressure minus the reduction in pressure necessary to float the fish gives a value here called the pressure of neutral buoyancy (Saunders, 1965). At the pressure of neutral buoyancy the gases in the swimbladder had expanded and increased the body volume to the point where the total body density equalled that of water. Assuming negligible differences in tissue density between individuals tested, the pressure of neutral buoyancy was an index of the buoyancy of the

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fish. The greater the pressure of neutral buoyancy the more buoyant was the fish. Fish observed to lose gas after the current was stopped were discarded. No fish was used twice.

The first series of tests was made to determine the time required for swimbladder adjustment to an increase in velocity. The tests were carried out on yearling parr (mean weight 18.5 g, range 12.0–28.0 g; mean fork length 11.5 cm, range 10.0–13.2 cm) between October 24 and November 25 at temperatures ranging from 9 to 11.3 C. Pressures of neutral buoyancy were determined on parr held for 3, 6, 12, and 24 hr in the flume at a flow of 8.3 cm/sec (Fig. 1A). A new group of 9–15 individuals was examined at each time interval. A similar series of tests was made on groups of 5–11 individuals held at the negligible flow of less than 1.5 cm/sec. At 12 hr ($P < 0.05$) and at 24 hr ($P < 0.01$), the mean neutral buoyancy values were lower for the group held in the more rapid flow (White ranking test for unequal sample sizes (Snedecor, 1956)). Figure 1B shows the development of the difference in neutral buoyancy between the fast and negligible flows. The response was probably not completed at 24 hr but a considerable response did occur within the first 12 hr.

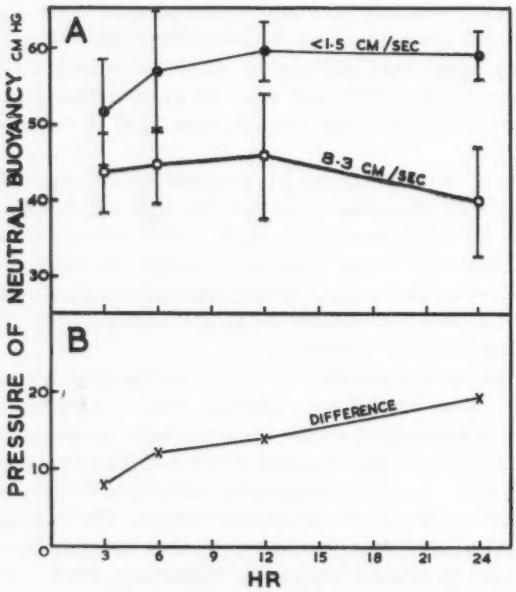

FIG. 1. A. The relationship between time and pressure of neutral buoyancy of yearling parr at velocities of <1.5 and 8.3 cm/sec. Two standard errors on either side of the mean are shown. B. The relationship between time and the difference in pressure of neutral buoyancy between parr held at <1.5 and 8.3 cm/sec.

In the second series of tests the relationship between neutral buoyancy and water velocity was determined. Both yearlings (mean weight 18.4 g, range 9.1–30.6 g; mean fork length 12.5 cm, range 8.6–15.0 cm) and underyearlings (mean weight 1.9 g, range 0.9–3.4 g; mean fork length 5.8 cm, range 3.9–7.2 cm) were used. Between October and late December the pressure of neutral buoyancy was determined on groups of yearlings and underyearlings held in the flume for periods of 12–18 hr at different velocities (Table I). Thus, the extent of buoyancy adjustment within the 12–18-hr period and not necessarily the completed response was determined for different current velocities.

At any given velocity the mean value for yearlings was not significantly greater than that for underyearlings. In tests I–IV which were carried out during October and early November, there was an inverse correlation between the pressure of neutral buoyancy (yearlings and underyearlings combined) and water velocity ($r = -.80$; df = 78; $P < 0.01$). It is apparent that at this season parr regulate their buoyancy by decreasing swimbladder volume in response to increments in current speed.

The values in tests V, VI, and VII, which were made during late November and December to check the previous findings, did not follow the earlier trend. At the higher velocities tested (VI and VII) parr were more buoyant ($P = 0.05$) than previously (II, III, and IV). Furthermore, test V suggested that in

TABLE I. Relationship between current velocity and pressure of neutral buoyancy of Atlantic salmon yearlings and underyearlings.

Test no.	Current (*cm/sec*)	Pressure of neutral buoyancy		Age	Test period	Temperature (*C*)	
		Mean	SE			Mean	Range
I	<1.5	58.9	3.09	yearling	Oct. 20–30	13.0	11.5–13.5
		57.5	2.90	underyearling			
II	5.9	46.0	4.64	yearling	Oct. 25–Nov. 5	12.0	11.5–12.5
		41.9	6.05	underyearling			
III	11.3	37.5	3.86	yearling	Oct. 23–Nov. 2	13.0	12.5–14.0
		32.1	4.21	underyearling			
IV	22.2	32.0	4.81	yearling	Nov. 11–21	10.6	10.0–11.5
		31.5	3.76	underyearling			
V	<1.5	54.2	5.33	yearling	Dec. 17–21	8.0	7.5– 8.5
		44.3	5.95	underyearling			
VI	5.9	62.1	1.62	yearling	Dec. 11–16	9.0	8.5– 9.5
		59.8	2.06	underyearling			
VII	17.0	48.3	3.60	yearling	Nov. 23–Dec. 31	9.6	9.0–10.0
		45.2	4.62	underyearling			

late December parr held in a negligible flow were less buoyant than parr similarly tested in October (I). Both the decreased buoyancy in a negligible flow and the decreased response to an increase in velocity in winter have been confirmed by more recent observations. It is evident that seasonal changes in buoyancy occur. Work is now in progress investigating seasonal changes in buoyancy more fully and attempting to determine the factors which govern the changes.

We are grateful to Dr J. L. Hart, Fisheries Research Board of Canada Biological Station, St. Andrews, for his interest in our work and for the loan of the experimental flume and vacuum pump. Mr D. MacLean, East Florenceville Fish Culture Station, Department of Fisheries of Canada, kindly provided us with fish through the courtesy of Dr R. R. Logie, Fish Culture Development Branch, Maritimes Area. Drs M. W. Smith and P. V. Dehadrai and Miss Lesley J. Pinder offered helpul suggestions for improving this presentation. The project was supported through grants-in-aid of research to J. G. Eales from the National Research Council of Canada.

Department of Biology
University of New Brunswick
Fredericton, N.B.

NANCY M. NEAVE
CAROLE L. DILWORTH
J. G. EALES

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

R. L. SAUNDERS

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Feeding and Growth of Redfish (Genus *Sebastes*) in Captivity

There has been a controversy among biologists studying redfish as to whether the fish grow quickly or slowly. The controversy arose mainly because of different criteria used in grouping zones in otoliths when age-reading. This resulted in different estimates of mean length-at-age. At a Redfish Symposium sponsored by the International Council for the Exploration of the Sea and the International Commission for the Northwest Atlantic Fisheries in 1959 the biologists present agreed to accept the concept of slow growth for *marinus* and *mentella* species (ICNAF Special Publication No. 3, 1961, p. 253). Personnel at this laboratory were interested in this problem and were in a convenient position to capture live fish from the Eastport, Maine, stock described by Kelly and Barker (1961), and to hold them to observe their growth.

Accordingly, in September 1960, 31 fish from this stock were taken by handline and brought to St. Andrews. They were kept under observation in a fibreglass tank measuring 6 ft in diameter by 4 ft in height. During the period of observation the numbers diminished from 31 to 15 due to deaths and to the removal of four fish on September 6, 1961, for use in another experiment. Conditions in the tank varied with those in the water in Passamaquoddy Bay, the water being constantly changed from the supply piped into the laboratory.

The redfish were fed frozen herring which were chopped into small pieces and dropped into the tank, a few at a time. Weights of food eaten were not recorded but the amount of feeding activity was. The fish were offered food until feeding activity ceased. Amount and frequency of feeding varied from 50 to 300 g of herring introduced at about 3-day intervals. Unused portions were usually removed the next day. At irregular intervals the fish were removed and measured to the nearest millimetre, fork length. Observations were taken for 22 months.

Temperature data and observations on feeding were averaged for each month. The seasonal change in water temperature from Passamaquoddy Bay was evident (Table I), fluctuating from a high of 13.4 to a low of 1.6 C. Observations of feeding activity indicated that during low-temperature periods very little food was consumed. Fish fed most actively when the water temperature was near the high point in the seasonal cycle.

The fish were measured nine times during the period of observation (Table I). There was some small variation in measurements due to mutilation of the ends of the rays in the caudal fin in some fish. Measurement data in Table I show that the average length of the fish changed very little over the 22 months. The mortality mentioned earlier did not seem to be selective for any particular size of fish since the mean length of 10 dead fish measured was only 1 mm above the average for the remainder at the time. Kelly and

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TABLE I. Feeding and growth of redfish in tanks.

Date	Temp.	Feeding	No. fish	Mean length	Length range
	C			*mm*	*mm*
Sept. 60	12.5	No feeding	31		
Oct. 60	11.2	Feeding fairly well	31		
Nov. 60	9.0	Feeding fairly well	31	223	180–271
Dec. 60	5.1	Little feeding	31	226	186–274
Jan. 61	3.1	Very little feeding	31	225	187–279
Feb. 61	1.7	Very little feeding	31		
Mar. 61	1.6	Very little feeding	31		
Apr. 61	3.9	Very little feeding	30	224	184–277
May 61	6.5	Started feeding	28		
June 61	9.3	Little feeding	27		
July 61	11.1	Feeding well	27		
Aug. 61	12.7	Feeding sporadic	27		
Sept. 61	13.4	Very little feeding	27	224	178–268
Oct. 61	12.1	Little feeding	20		
Nov. 61	10.4	No feeding	17		
Dec. 61	7.6	No feeding	16	223	175–267
Jan. 62	4.8	No feeding	16	224	178–270
Feb. 62	2.8	No feeding	15		
Mar. 62	3.9	Very little feeding	15		
Apr. 62	4.9	Very little feeding	15		
May 62	7.2	Very little feeding	15	218	177–248
June 62	9.9	Feeding increasing	15		
July 62	11.5	Feeding well	15	227	187–255

Barker (1961) found a very slow growth rate for redfish from this population, about 15–20 mm per year for untagged fish and less than 8 mm in 2 years for tagged individuals. Our experience with feeding other groundfish species and recording their growth is that they grow at a rate corresponding to that in natural surroundings (Kohler, 1964).

The fish remaining on July 17, 1962, were killed. Examination showed that there were 12 females and 3 males, all of which were immature. Twelve of the 15 fish had food either in the stomach or in the intestine.

Otoliths from the fish were taken and examined by two readers. Of their readings, 5 out of 15, agreed, and 10 out of 15 differed by 1 year. Readings indicated that there were six 5-year-old, four 6-year-old, and five 7-year-old fish in the group. In many of these otoliths the last one or two hyaline zones, presumably formed while the fish were in the tank, were noticeably darker than the ones closer to the otolith centre.

Particular appreciation is expressed to F. Purton who looked after the fish and recorded their feeding activity.

Fisheries Research Board of Canada A. C. KOHLER
Biological Station, St. Andrews, N.B.

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Efficiencies of the Net Plankton and the Centrifuged Seston Methods in Highly Eutrophic Lakes

Birge and Juday (1922) showed that in lakes investigated in Wisconsin the organic weight biomass of net plankton (no. 20 silk) ranged from one-third to one-twelfth that of the centrifuged seston. They suggested that the usual range was one-third to one-tenth. In spite of its shortcomings, the tow net remained in use (Rawson, 1953) to estimate plankton biomass in lakes. Discussions with various investigators have revealed that the net plankton method is still in use in both qualitative and quantitative plankton studies.

Since it is very difficult to compare the data in the literature on the net plankton and centrifuged plankton methods, the two methods were used simultaneously in a study of four lakes (Table I) in central Alberta to see how they compare in highly eutrophic lakes. A description of the lakes will be published elsewhere (Kerekes and Nursall, 1966, in press).

Hortobágyi (1962) compared algal cell counts obtained by a plankton net (no. 25) with those obtained by dipping a bucket directly into the water. He found that a marked difference occurs in both quantity and quality of algal cells in a unit water volume taken by the two methods.

Net plankton samples and water for centrifuged plankton (seston) samples were collected at the same time from an anchored boat in the open part of each of the four lakes investigated. Two samples of each type were collected at one sampling. Net plankton samples were collected in a 25-cm diameter Wisconsin-type plankton net (no. 20 bolting silk) by pouring 10 gal of surface water through the plankton net with a 2-gal pail. To minimize error in weight due to different total dissolved contents in different lakes, the samples were

TABLE I. Mean values by net plankton and seston methods for summer organic matter biomass of the surface water in five bodies of water in central Alberta in 1964.

Lake	Net plankton (*mg/liter*)	Seston (*mg/liter*)	Net plankton: seston
Antler	3.5	16.8	1:4.8
Cooking	1.1	71.1	1:64.6
Hastings	3.0	11.8	1:3.9
Ministik Area 1[a]	0.5	22.5	1:45.0
Ministik Area 2[a]	2.9	13.9	1:4.8

[a]Ministik Lake is a multibasin lake, formed by several distinct basins connected by narrow channels.

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washed thoroughly with distilled water in the net bucket before being transferred to a specimen bottle. Samples were preserved in 70% alcohol.

Hartman (1958) suggested that in highly eutrophic lakes at least three runs are necessary through the Foerst centrifuge to obtain a reasonably accurate estimate of seston in lake water. Following his procedure four samples of water from each lake were centrifuged repeatedly six times at the recommended rate, with gravimetric determinations being made on the residue from each run. The removal of seston with three centrifugings was only 87–91% efficient. To compensate for this error 11% was added to each seston weight in later determinations (obtained by three centrifugings) as suggested by Hartman (1958). Water samples for quantitative seston study were taken just below the lake surface with a 1200-ml Kemmerer bottle and were transferred into 2-quart bottles. The bottles were kept in darkness in a cooler chest. Upon return to the laboratory, 1 liter of water from each sample was centrifuged three times in a Foerst continuous-flow centrifuge for 6–7 min each centrifuging. To minimize error in weight due to different total dissolved contents in different lakes, 30 ml of distilled water was passed through the centrifuge at the end of each run. The seston samples were transferred to small glass vials, and preserved in 70% alcohol.

Both seston and net plankton samples were dried 24 hr at 100 C in porcelain crucibles, weighed, then ashed at 600 C for 30 min, and weighed again on a Mettler, type H6 electrical balance. The samples were kept in a desiccator until weighing.

The ratios (Table I) of the mean net plankton (np) values to the seston (s) values for the summer organic matter biomass ranged from 1:3.9 (Hastings Lake) to 1:64.6 (Cooking Lake). The same ratio for individual samples (Fig. 1) ranged from 1:1.2 in Ministik Lake Area 2 (July 18) to 1:103.8 in Cooking Lake (August 6), representing 86-fold variation in efficiency. The efficiency of the net plankton method in relation to that of the seston method varied greatly for different samples in Antler Lake. The np:s ratio was 1:1.7 on June 3, 1:2.6 on July 12, 1:12.7 on July 29, 1:6.5 on August 15, and 1:14.2 on September 7. In Antler Lake the net plankton method indicated a continuous drop in plankton standing stock, yet the centrifuge method showed an overall increase (Fig. 1A).

The efficiency of the net plankton method as compared to that of the plankton centrifuge method varied from lake to lake and varied within a lake throughout the season. The great variation in efficiency in different samples was caused by the different qualitative composition of the phytoplankton. In Cooking Lake the phytoplankton is composed mainly of smaller forms which pass through the meshes of the plankton net. Consequently, the np:s ratio is very low (Fig. 1B). A similar situation occurred in Ministik Lake Area 1. In Area 2 of this lake a bloom of *Microxystis aeruginosa*, which is a colonial form large enough to be retained by the plankton net, was at peak on July 18. Because the efficiency of the plankton net increases when larger forms are present, the net plankton method showed (Fig. 1D) a sudden increase,

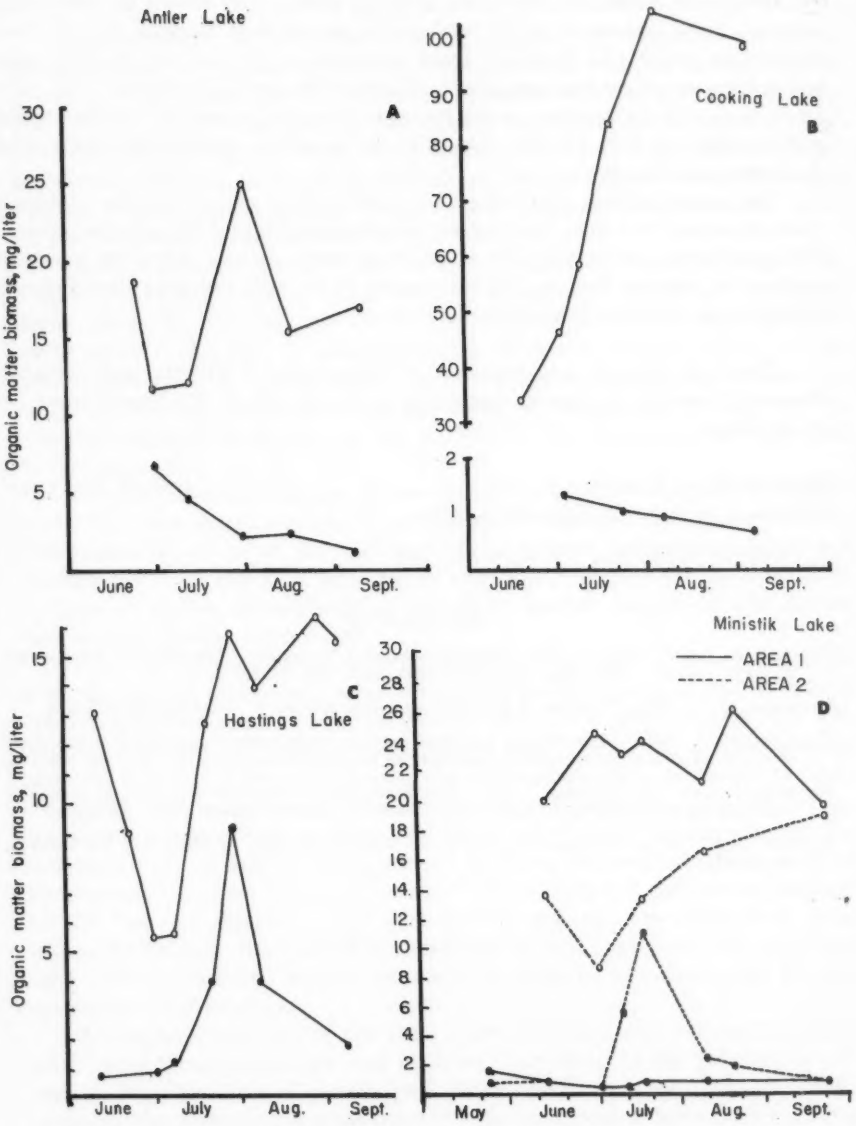

FIG. 1. The relations between the seston and net plankton values for summer organic matter biomass of surface water in four lakes in central Alberta, 1964. ○ — seston; ● — net plankton.

then a decline, of biomass in July and August, reflecting the intensity of the *M. aeruginosa* bloom, not the total biomass present. As shown by centrifuge samples, the biomass of seston had a slow continuous increase for the corresponding period. In Hastings Lake the relation of the net plankton and seston biomass values was comparable to that in Ministik Lake Area 2 (Fig. 1C). In Antler Lake an increase in the biomass of seston caused by smaller forms was recorded on July 29 (Fig. 1A). The net plankton method showed a drop in biomass on that day.

The net plankton method gives a misleading picture of the plankton biomass present in a lake, particularly when phytoplankton blooms are present. The error of this method further increases in vertical hauls, when the plankton retained by the net tend to clog the meshes of the silk, reducing the efficiency of the net as it moves toward the surface.

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Department of Zoology
University of Alberta, Edmonton, Alta.

JOSEPH KEREKES[1]

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[1]Present address: Canadian Wildlife Service, Dept. of Northern Affairs and National Resources, Ottawa, Ontario.

Blood Lipids of the Lobster, *Homarus americanus*

Approximately 70% of the lobsters captured by Canadian fishermen are shipped live to consumer outlets. This operation requires the commercial storage of large quantities of live lobsters often for extended periods (McLeese and Wilder, 1964) and periodically heavy mortalities occur among these stored lobsters. Many of these losses are the result of a septicemic disease, gaffkaemia (Roskam, 1957), caused by infection of the lobster by the bacterium *Gaffkya homari* (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947; for further references see Stewart et al., 1966).

Heavily infected lobsters suffer a disappearance of the hemocytes, prolonged hemolymph clotting times, reduced hemolymph viscosity, and finally death (Snieszko and Taylor, 1947). The infected lobster becomes progressively weaker and can, if wounded, bleed to death through failure of the hemolymph to clot. Consequently, a knowledge of the normal lobster hemolymph clotting mechanism and the mode of interference with it by the infective agent is important to the studies of this disease being conducted at this laboratory.

The importance of certain plasma phospholipids in blood clotting mechanisms has been shown already for a number of animals (Troup et al., 1961). Unfortunately, data on the composition of lobster hemolymph lipids are unavailable. This report is intended to supply information on the lipid composition of lobster hemolymph as an aid to further studies on the lobster hemolymph clotting mechanism.

Two liters of hemolymph were collected by drawing 5- to 10-ml quantities from the ventral abdominal sinus of each lobster of a group of approximately 300 brought ashore 6 hr earlier on June 1, 1961, at Pictou County, N.S. The individual weights of these lobsters ranged from 225 to 350 g. Immediately after its withdrawal from the lobster the hemolymph was transferred to flasks containing the extraction solvents and mixed thoroughly. The total lipid was extracted according to Bligh and Dyer (1959) by homogenizing the hemolymph with 5.0 liters of methanol and 2.5 liters of chloroform. The resulting homogenate was then thoroughly mixed with 2.5 liters of chloroform followed by 2.5 liters of distilled water with further mixing. The chloroform layer was quantitatively recovered by centrifugation and contained 4.61 g of total lipid. This represented a lipid content of 2.3 mg/ml of hemolymph for this particular lot of lobsters.

A sample (1160 mg) of the total extracted lipid was fractionated using silicic acid chromatography and analyzed according to the procedures previously described for cod muscle lipid (Bligh and Scott, 1966). The chromatograph in Fig. 1 shows the separation of eight components from the total lipid. Analytical data on the various components are summarized in Table I.

The results of this study have shown that phospholipids make up approximately 65% of the lipids in lobster hemolymph, phosphatidyl choline and

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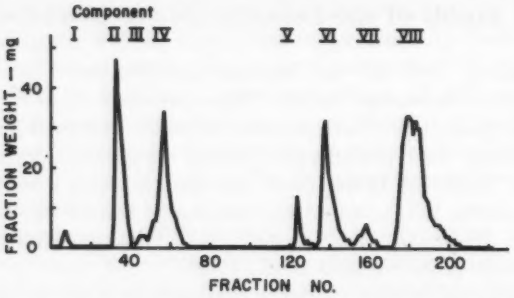

FIG. 1. Silicic acid column chromatographic separation of lipid components from lobster hemolymph. Total lipid applied, 1160 mg; components I–IV eluted with increasing gradient of ether in hexane; components V–VIII eluted with increasing gradient of methanol in chloroform; 10-ml fractions collected.

TABLE I. Analytical data on lipid components from lobster hemolymph.

	Component	Weight (*mg*)	Per cent of total lipid	Analytical data
I	Hydrocarbon	13	1.1	No sterol
II	Triglyceride	180	15.5	No sterol; no free fatty acid
III	Free fatty acid + sterol	28	2.4	12 mg free fatty acid
IV	Sterol	181	15.6	75 mg cholesterol (41.5%)
V	Unidentified phospholipid	35	3.0	0.37 mg N; 0.58 mg P
VI	Phosphatidyl ethanolamine + phosphatidyl serine (about 6:1)	201	17.3	3.23 mg ethanolamine-N; 0.54 mg serine-N
VII	Phosphatidal ethanolamine + phosphatidyl serine (about 2:1) + trace inositol phosphatide	37	3.2	0.46 mg ethanolamine-N; 0.24 mg serine-N
VIII	Phosphatidyl choline + sphingomyelin (about 5:1)	482	41.6	73.4 mg total choline; 61.3 mg lecithin choline
	Recovery	1157	99.7	

phosphatidyl ethanolamine being the major constituents. The neutral lipids were mainly triglycerides and free sterols, each representing about 15% of the total lipid. No esterified sterols were detected.

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Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

E. G. BLIGH[1]
MARGARET A. SCOTT[2]

[1]Present address: Fisheries Research Board of Canada, Office of the Chairman, Ottawa 8, Ont.
[2]Present address: 23 Bayshore Drive, Ottawa 14, Ont.

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